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Review

On the epigenetic origin of cancer stem cells

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ABSTRACT

Epigenetic mechanisms are the key component of the dynamic transcriptional programming that occurs along the process of differentiation from normal stem cells to more specialized cells. In the development of cancer and according to the cancer stem cell model, aberrant epigenetic changes may ensure the property of cancer cells to switch cancer stem cell markers on and off in order to generate a heterogeneous population of cells. The tumour will then be composed of tumourigenic (cancer stem cells) and non-tumourigenic (the side population that constitutes the bulk of the tumour) cells. Characterizing epigenetic landscapes may thus help discriminate aberrant marks (good candidates for tumour detection) from cancer stem cell specific profiles. In this review, we will give some insights about what epigenetics can teach us about the origin of cancer stem cells. We will also discuss how identification of epigenetic reprogramming may help designing new drugs that will specifically target cancer stem cells.

Contents

1. Introduction—what is epigenetics?

Epigenetics define heritable changes in the pattern of gene expression that are not mediated by alterations in the primary nucleotide gene sequence [1]. Among these stable but reversible changes, DNA methylation and specific post-translational modifications on NH_2-terminal histone tails are key mechanisms that control chromatin compacting. DNA methylation is almost entirely limited to CpG dinucleotides while histone modifications include a variety of modifications including acetylation, methylation, phosphorylation, ubiquitination, biotinylation, and SUMOylation. Crosstalks between the numerous writers of the epigenetic code (*i.e.* DNA methyltransferases, histone acetyltransferases, deacetylases, methyltransferases, demethylases, etc.) define whether genes are activated or repressed [2]. Acetylation of histones H3 and H4 is mostly associated with gene expression. On the contrary, DNA methylation, di- and trimethylation of H3 lysine 9 (H3K9) and trimethylation of H3K27 elicit the compaction of chromatin leading to gene silencing through the recruitment of heterochromatin protein 1 (HP1) and polycomb group (PcG) proteins.

Adding a new layer of complexity to the epigenetic machinery, microRNAs (miRNA) known to target the 3′-untranslated regions (3′-UTR) of mRNA transcripts for degradation or translation repression [3,4], have also recently been shown in human cells to trigger transcriptional silencing *via* chromatin remodelling [5].

Abbreviations: ESC, embryonic stem cell; CSC, cancer stem cell; HP1, heterochromatin protein 1; PcG, polycomb group protein; HDAC, histone deacetylase; DNMT, DNA methyltransferase; BMI-1, B cell Moloney murine leukemia virus Insertion region

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Epigenetic changes are increasingly regarded as key events in the development of cancer. Aberrant DNA methylation profiles (large-scale hypomethylation [6,7] associated with specific, non-random, hypermethylation of CpG islands [8]), histone modification landscapes and miRNA signatures are early features of carcinogenesis occurring in preneoplastic lesions as well as in adjacent tissues. This has led to the hypothesis that epigenetic changes may predispose cells to malignant transformation or even constitute one of the primary transforming events.

2. The role of epigenetic changes in normal embryonic and adult stem cell differentiation

Both embryonic development and stem cell differentiation in adults involve crucial changes in gene expression profiles. Genes specific for each cell fate are activated while pluripotency factors are silenced, most likely by specific epigenetic rearrangements [9,10].

Embryonic stem cell (ESC) status involves multiple layers of molecular events designed to impose a flexible but precise control over the expression of genes important for development [11]. Several factors of pluripotency and self-renewal have been identified, including OCT4, NANOG, SOX2, FOXD3, REX1, KLF4, c-myc, Lin-28 and the Wnt signalling pathway [12,13].

Along with the expression of these specific factors, ESCs harbour a particular epigenetic landscape with a highly dynamic and permissive chromatin. This state is characterized by a distinct DNA methylation profile, the genome of ESC being globally less methylated than differentiated somatic cells, despite a high level of Dnmt3b expression [14,15]. Structural proteins such as HP1 and histone H1 also bind more loosely to chromatin compared to differentiated somatic cells [16,17]. These features are essential for chromatin rearrangements and therefore for global transcriptional programming upon differentiation. More particularly, developmentally regulated genes are characterized by the presence of both active and repressive histone marks. In that case, large genomic regions with trimethylated H3K27 colocalize with smaller regions containing permissive chromatin marks such as methylated H3K4. This chromatin bivalency allows genes encoding specific transcription factors or early differentiation factors to be prepared for rapid transcriptional activation upon differentiation [18].

PcG complexes, on the other hand, act to stabilize a repressive chromatin structure and have been shown to play important roles in the direct silencing of cell differentiation regulators such as Otx2, Satb2 and Tbx3 factors [19]. Among the actors of PcG complexes, the B cell Moloney murine leukemia virus insertion region protein BMI-1 serves as a key regulatory component and plays a critical role in the self-renewal of adult stem cells.

Many miRNAs are specifically expressed during differentiation and embryogenesis. Indeed, ESCs express a unique set of miRNAs [20]. Moreover, some miRNA precursors (pre-miRNAs) expressed in stem cells are not processed and cleaved into mature miRNA until differentiation is initiated [21]. This strongly suggests that miRNA may have a role in the maintenance of the pluripotent cell state and in the regulation of early mammalian development.

Upon differentiation, ESCs see their epigenome rich in euchromatin evolve toward a more compact heterochromatin structure. A unique DNA methylation profile is acquired, involving methylation in domains mostly found in regions adjacent to CpG islands, named CpG island shores [22,23]. Acquisition of this specific profile reduces cell pluripotency and induces progressive activation of cell- and tissue-specific genes [9]. Heterochromatic markers, such as HP1 proteins modify their localization from a hyperdynamic to a more concentrated state, stabilizing the new cell fate. Thus, the chromatin becomes progressively less permissive. This is characterized by a global decrease in active histone marks such as acetylated histones H3 and H4 [16] and by a specific increase in silencing-associated di- and trimethylated H3K9 [24]. Additionally, bivalent chromatin profiles are changed either to methylated H3K4 at genes specific for the particular cell fate in which they are engaged or to trimethylated H3K27 at genes specific for other cell fates [18].

For instance, Oct4 and Nanog promoters shift from H3K4me3 to H3K27me3 marks as cells differentiate [25] and undergo rapid DNA methylation upon differentiation *in vitro* [13] whereas they are unmethylated in undifferentiated human ESC [26]. Conversely, Oct4 and Nanog have been shown to interact with each other and associate with Hdac1/2 and Metastasis Associated 1/2, in a complex named NODE (Nanog and Oct4 associated DEacetylase). This complex contains HDAC activity comparable to NuRD complex [27], hence participating in the epigenetic landscaping specific for stemness. On the contrary, because of changes in DNA methylation and histone modifications in regions enriched for their binding sites, these factors can no longer bind the promoters of their target genes in differentiated cells [11,28].

Epigenetic mechanisms are thus a key component of the dynamic regulation of differentiated *versus* stem cell identities.

3. The cancer stem cell hypothesis and the establishment of an epigenetic tumour hierarchy

The clonal model of carcinogenesis, in which genetic and epigenetic changes occur over time to confer a selective growth and survival advantage to individual clones within a tumour, has long prevailed. However, numerous studies conducted in hematologic malignancies as well as in solid tumours have shown that only a proportion of the cancer cells are able to initiate new tumour growth when xenografted in immunodeficient NOD/SCID mice. The percentages of tumour-initiating cells vary from 0.0001% [29] to 25% [30] depending on xenotransplantation assay conditions. In most human cancers (melanomas, sarcomas, blood, brain, lung, gastrointestinal, hepatocellular, prostate, breast and ovarian cancers), a panel of candidate markers has been described that helps distinguish between the relatively rare subpopulations of tumourigenic cells from non-tumourigenic (side population) cancer cells (Table 1; [29]).

This observation has led to the cancer stem cell (CSC) model in which tumours would arise from rare to moderately frequent cells able to initiate a hierarchy of proliferative and progressively differentiating non-tumourigenic cells. This non-tumourigenic population of cells would then form the bulk of the tumour. In this model, CSC would have similar properties to normal adult stem cells or ESC. These properties would include (i) the capacity of self-renewal potentially through asymmetric division, (ii) the use of key regulatory pathways and (iii) the establishment of dynamic epigenetic profiles. These profiles would potentially help discriminate CSC from non-tumourigenic cancer cells. Hence, the tumour hierarchical organization would be comparable to that found in normal tissue, where epigenetic changes trigger both cell differentiation and cell fate. CSC, however, are thought to harbour higher proliferative rates and be capable of causing constant expansion of existing tumours or forming new tumours through metastatic processes. Moreover, the dependence to the stem cell niche may provide a tremendous difference between normal stem cells and CSC. In that case, the normal stem cell niche would control the balance between cell proliferation, self-renewal and differentiation [31], while the CSC niche would govern metastatic colonization [32].

Since clonogenicity implies that cancer cells accumulate selective mutations over a relatively long period of time [33,34], it is rather unlikely that genetic changes throughout a neoplastic clone would result in rapid and extensive proliferation limited to only a subpopulation of cancer cells. Hence, differences between tumourigenic and non-tumourigenic cancer cells may rather reside in their epigenetic profile. Similar to stem cells giving rise to the panel of differentiated cells in each organ, it is thought that CSC may give rise to the cellular heterogeneity of the tumours by initiating epigenetic reprogramming (Fig. 1). Confirming

Table 1
Epigenetic regulation of cancer stemness markers.

Gene	Tumour type	CSC profile	Epigenetic regulation/*function*	Reference
CD34	AML	CD34+/CD38-	415 bp CpG island[a]	
CD38	Childhood B-ALL	CD133+/CD19−/CD38−	731 bp CpG island[a]	
CD19	ALL and CML-BC	CD34+/CD38−/CD19+ t(12;21)	Chromatin remodeling	[74]
CD24	Breast carcinoma	CD44+/CD24$^{-/low}$/Lin	No differential methylation between CD44+CD24− and CD44+CD24+ breast cancer cells	[75]
CD44	Head and neck cancer, prostate, gastric and colorectal carcinoma	CD44+	DNA methylation	[76]
Thy-1	Liver cancer	CD90+	DNA methylation	[77]
ENG	Renal carcinoma	CD105+		
CD117 (c-kit)	Ovarian adenocarcinoma	CD44+/CD117+	DNA methylation and miRNA	[78,79]
Prominin-1	Brain tumour, glioblastoma, osteosarcoma, Ewing's sarcoma, endometrial, hepatocellular, colon and lung carcinomas, ovarian and pancreatic adenocarcinoma	CD133+	Histone modifications and DNA methylation	[28,80–82]
Bmi-1	Glioblastoma	CD133+/Bmi1+	*Polycomb group protein*	[83]
ALCAM	Colorectal carcinoma	EpCAM^{hi}/CD44+/CD166+	DNA methylation	[84]
NGFR	Melanoma	CD271+	DNA methylation	[85]
ALDH1	Colorectal, breast, prostate and squamous cell carcinomas, pancreatic adenocarcinoma, osteosarcoma	ALDH1+	DNA methylation	[86]
EpCAM (ESA)	Colon carcinoma and Pancreatic adenocarcinoma	EpCAM^{hi}/CD44+ and CD44+/CD24^{+}/EpCAM+	DNA methylation	[87]
ASCL1	Lung carcinoma	CD133+/ASCL1^{hi}/ALDH^{hi}	Histone modifications	[88,89]
JARID1B	Melanoma		*Histone demethylase*	[90]
PDPN	Squamous cell carcinoma	Podoplanin+	DNA methylation	[91]
TACSTD2	Prostate carcinoma	TROP2+/CD133+	DNA methylation	[92]

[a] CpG island information corresponds to the prediction of the UCSC Genome Browser on Human Mar. 2006 (NCBI36/hg18) Assembly with the following criteria: GC content of 50% or greater, length greater than 200 bp, ratio of observed number of CG dinucleotides to the expected number on the basis of the number of Gs and Cs in the segment greater than 0.6.

this hypothesis, the lower level of DNA methylation and repressive histone marks such as H3K27me3 at tumour suppressor genes have been found in putative breast and liver CSC compared to their "differentiated" counterparts. Therefore, in these tumours, CSCs harbour a higher transcriptionally active chromatin state than "differentiated" cancer cells [35]. Similarly, histone modifications such as the marker of activated gene H3K4me3 have been found predominantly in CSC of AML patients at genes characteristic for stemness, including Bmi1, Notch1 and Wnt1 [27]. In head and neck squamous cell carcinomas (HNSCC), CD44+ CSCs also show a unique epigenetic signature of 22 genes with a significant distinct methylation pattern compared with CD44−non stem cells [36].

The dynamic property of cancer cells to switch CSC markers off (upon cell proliferation shown by the heterogeneity of the tumour cell population [37]) and back on (as described in experimental models [38]) supports an epigenetic mechanism. Hence, virtually all known CSC markers have been shown to be regulated either by DNA methylation or histone modifications (Table 1), or themselves are chromatin modifying enzymes (such as BMI-1 or JARID1B [39]). Additionally, miR-200c, miR-203 and miR-183, also play an important role in the regulation of stem cell markers, inducing differentiation in pancreatic and colorectal CSC [40]. Consistent with these findings, loss of these stemness-inhibiting miRNA has been shown to lead to the establishment of epigenetic patterns required for the formation and maintenance of breast CSC [41]. Signatures of CSC have therefore been recently established in breast as well as in liver cancers [42,43].

Further analyses will be crucial (i) to understand how this potential hierarchy is established and (ii) to discriminate CSC from non-tumourigenic cancer cells based on their epigenetic landscapes. Aberrant DNA methylation and histone marks that have been described in cancer for the past two decades may represent marks present in the bulk of the tumour and as such could be considered as good candidate markers for tumour detection. On the other hand, epigenetic profiles that are specific for CSC may resemble the original cancer-initiating cell, whether it is a dedifferentiated or a normal stem cell, and could be considered as good therapeutic targets.

4. The origin of CSC: what may we learn from epigenetic landscapes?

Normal adult stem cells are characterized by the capacity to self-renew. Their longevity in the stem cell niche allows normal stem cells, or their immediate progeny, to be the first targets of malignant transformation *via* accumulation of aberrant epigenetic profiles or genetic mutations [44–46]. Hence, improper activation of the normal self-renewal machinery could be a critical event in cancer initiation. Supporting the hypothesis of normal stem cells being the predecessors of CSC, lineage-tracking studies in the colon have shown that long-lived intestinal stem cells are susceptible to cancer-causing mutations [45,47]. Additionally, genes that are targets of PcG proteins and therefore known to be suppressed in normal stem cells show hypermethylation correlated with aging. This locks the cells in an undifferentiated state, therefore making it the first potential step toward malignant transformation [48].

Finally, gene expression signatures reminiscent of ESC have been found in various types of cancer especially poorly differentiated cancers, and markers of CSC are usually similar to normal stem cell markers in different organs [49]. For instance, Bmi1 has been described as a marker of both adult normal stem cells and CSC in colon, breast and prostate, as well as in hematopoietic and neuronal stem cells. In breast cancer, this key regulatory component of the Polycomb Repressive Complex-1, which regulates stem cell proliferation and blocks terminal differentiation to more committed cells through regulation of the Hedgehog pathway, is also increased in tumour-initiating cells [50].

Nevertheless, it is also interesting to note that CSCs differ from normal adult stem cells in that they do not retain the bivalent chromatin pattern [35]. Additionally, studies analyzing the DNA methylation profile of CSC indicate that epigenetic markers of stemness would resemble the ESC (expression of Dnmt1 and Hdac1) rather than the adult normal stem cell pattern (expression of MLL family of histone methyltransferases) [51].

Hence, the hypothesis of cell reprogramming should definitely not be excluded. Indeed, while cellular differentiation has been thought to be unidirectional, several recent studies have shown

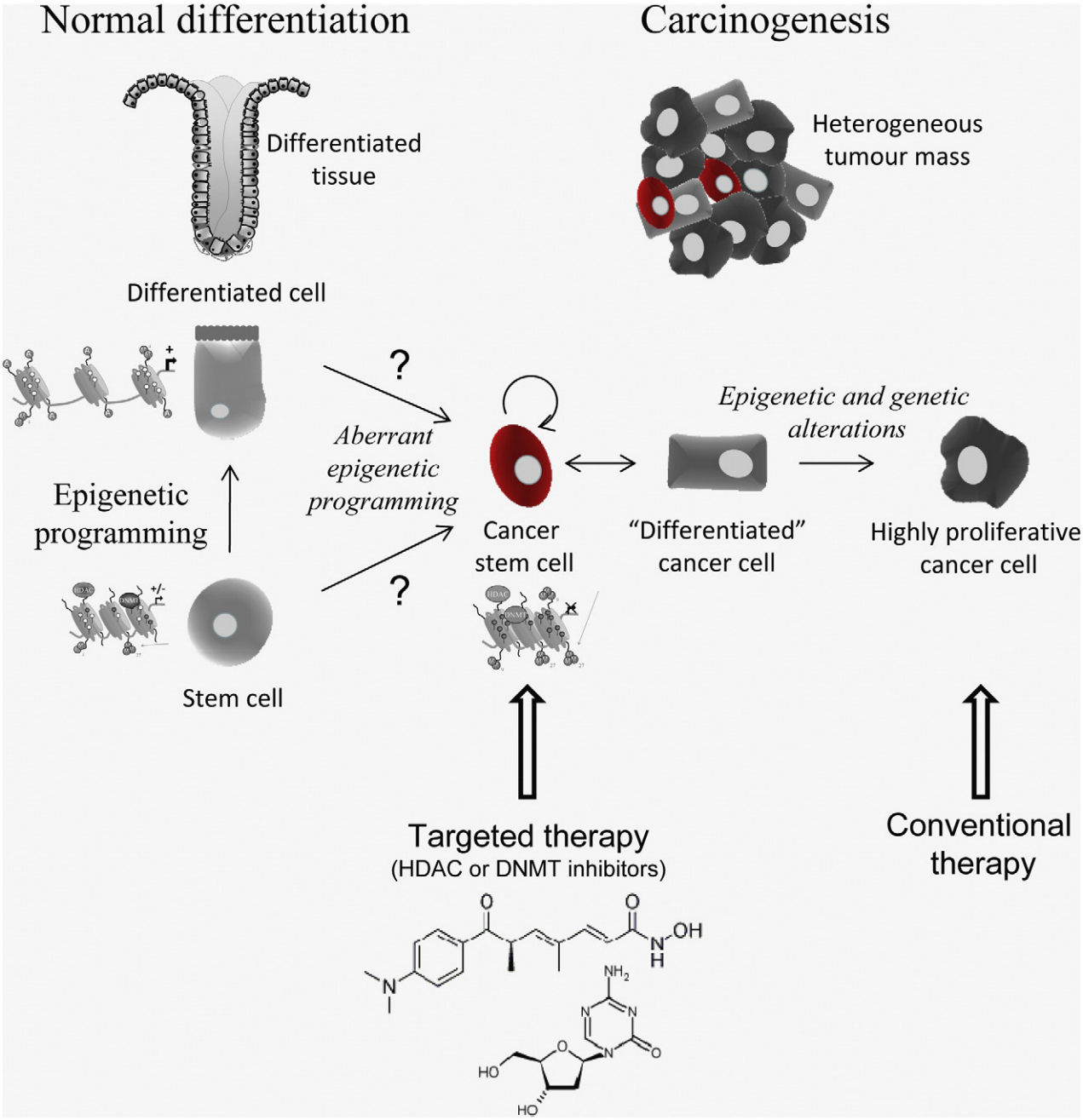

Fig. 1. Normal stem cells *versus* CSC, an epigenetic change. In this simplified model, aberrant epigenetic programming would trigger either the reactivation of self-renewal in differentiated cells or the initiation of transformation in normal stem cells to give rise to CSC. CSC would in turn give rise to relatively differentiated cancer cells that may acquire additional epigenetic and genetic alterations and lead to highly proliferative cancer cells. CSC, differentiated cancer cells and highly proliferative cancer cells would constitute the heterogeneity of the tumour. Targeted therapy may force CSC to reenter the normal process of differentiation and could represent a relevant addition to conventional therapies that only target highly proliferative cancer cells.

that the reexpression of only four genes encoding the transcription factors c-Myc, Oct4, Sox2 and Klf4 is sufficient to generate pluripotency. Expression of these factors can convert a terminally differentiated cell into a pluripotent cell by erasing the epigenetic pattern associated with cell differentiation. Therefore, epigenetic reprogramming that induces the expression of pluripotency factors in differentiated cells would lead to aberrant stemness and cancer. Supporting this hypothesis, Oct4, c-myc, Sox2 and Klf4 have all been shown to be regulated by epigenetic mechanisms in differentiating [52–54] or neoplastic cells [55]. More recently, Chaffer and collaborators have discovered a striking property of differentiated epithelial breast cells that are able to dedifferentiate and acquire stem-like properties, unveiling a noticeable degree of plasticity of non-stem cell compartments [38].

While mouse models and *in vivo* experiments such as xenotransplantation assays have brought great insight into the deciphering of the mechanisms of cancer cell growth and metastasis, it is still impossible, in patients, to retrospectively identify the cell-of-origin of naturally occurring cancers. Therefore, it is yet unknown whether CSCs originate from normal adult stem cells, more mature progenitors or even from differentiated cells. As discussed above, there is evidence for each model. However, epigenetic events are more likely to represent ideal dynamic changes for cells that enter a carcinogenetic path. Indeed, according to the "epigenetic progenitor model of cancer" proposed by Feinberg and collaborators early epigenetic disruption of progenitor cells would disturb the normal balance between undifferentiated and committed cells [56]. The epigenetic progenitor landscape would therefore be

reminiscent of the cell-of-origin while the bulk of the tumour would acquire new epigenetic and genetic alterations. The nature of epigenetic alterations involved in CSC generation may strongly depend on the normal cell phenotype from which they arise and cancers would acquire different characteristics according to their origin [57]. Supporting this hypothesis is the fact that transformation of genetically identical but phenotypically different human breast epithelial cells leads to distinct tumour types [58]. This indicates that epigenetic presetting greatly influences the type of generated tumour.

Therefore, epigenetic landscapes, if they are maintained through CSC self-renewal, would more likely indicate the exact origin of CSC. Confirming this hypothesis, symmetric DNA methylation patterns have proven to be very informative to deduce the clonal expansion dynamics of CSC and the mechanisms of tumour hierarchy in colorectal cancers [59].

5. Implications for cancer therapy

CSCs are more and more considered as the roots of cancer and therefore identifying and targeting CSCs for elimination appears as critical for cancer therapy. Most currently used cancer therapies target fast growing cells. If CSC share, as it is thought, the more slowly cycling characteristics of their normal counterparts, known treatments would rather lead to an enrichment in CSC. Recent studies conducted in epithelial cancers and in melanomas have indeed shown that CSC candidates harbour lower proliferation rates than the bulk of the tumour [39,60–62]. Thus, they are mostly resistant to current treatments, including radiotherapy and conventional chemotherapy [63–65]. Differentiation therapy through epigenetic drugs, such HDAC or DNMT inhibitors, represents however a particularly interesting and promising approach, as it is known that CSCs keep plasticity for differentiation [65]. For instance, the demethylating agent 5-aza-2′-deoxycytidine has been shown to inhibit self-renewing population of CD34+ cells [66].

These epigenetic drugs have proven valuable as new weapons against cancer, especially leukemia. However, their broad spectrum of inhibition, targeting whole families of enzymes may not be perfectly suited to most solid tumours. This is especially true for the specific targeting of CSCs which harbour a peculiar epigenetic landscape with the expression of specific epigenetic regulators or enzymes when compared to the rest of the tumour. More targeted epigenetic-based therapies may thus greatly improve patient outcome, as each epigenetic modifier enzyme could be a potential individual target for therapeutic manipulation [44].

For instance, Bmi-1 is currently considered as a potent novel target for drug discovery [67]. It has been shown that Bmi-1 silencing, either by siRNA or epigenetic drugs such as HDAC inhibitors (sodium butyrate or valproic acid), enhances the sensitivity of cancer cells to chemoradiation and inhibits tumour growth [68]. Additionally, therapies using properties of miRNA to specifically inhibit stemness effectors and CSC markers have provided very promising results. For example, miR-34a inhibits prostate CSC and metastasis by directly repressing CD44 [69], while miR-128 inhibits BMI1 in breast cancer and gliomas [70,71], miR-34a specifically targets Notch in pancreatic cancer [72], and miR-199b-5p negatively regulates HES1 in medulloblastomas [73].

Identifying the epigenetic reprogramming that occurs during the acquisition of CSC properties and understanding the differences between normal adult stem cells and CSC will therefore help designing new drugs that will specifically target CSC without altering tissue homeostasis.

6. Conclusion and perspectives

A new challenge in medical science is to determine the origin of cancer resistance to most current therapies. Evidence have been brought that in the battle against cancer, CSC, if they exist, and non-CSC will both need to be targeted with specific strategies to eliminate both the bulk of a tumour and its potential roots. However, as most studies have focused on entire tumours with heterogeneous cell populations, our knowledge on human CSC, a small population of cells so far only identifiable by their capacity to generate new tumours in immunodeficient mice, remains very limited. This issue will more likely be overcome with the use of new tools such as next generation sequencing that allows genome-wide epigenetic profiling of a single stem cell. Hence, defining the early epigenetic reprogramming or deprogramming that gives rise to the cancer cell population instead of normal differentiated cells will be crucial for the discovery of new therapeutic strategies of cancer.

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