Epigenetic mechanisms driving tumor supportive microenvironment differentiation and function a role in cancer therapy?

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Abstract

The tumor microenvironment (TME) plays a central role in tumor development and drug resistance. Within TME, the stromal cell subset, called cancer-associated fibroblasts (CAFs), is a heterogeneous population originating from poorly characterized precursors. Since CAFs do not acquire somatic mutations, other mechanisms like epigenetic regulation, could be involved in the development of these cells and in the acquisition of tumor supportive phenotypes. Moreover, such epigenetic modulations have been correlated to the emergence of an immunosuppressive microenvironment facilitating tumor evasion. These findings underline the need to deepen our knowledge on epigenetic mechanisms driving TME development and function, and to understand the impact of epigenetic drugs, that could be used in future to target both tumor cells and their TME.

KEY WORDS: Epigenetic, Cancer, Microenvironment, Cancer-Associated Fibroblasts

I) Introduction

Epigenetic mechanisms

The concept of epigenetics emerged in the middle of the 20th century, with Conrad Waddington; who proposed the word, 'epigenetic', to describe biological cell diversity that cannot be attributed to alterations in the deoxyribonucleic acid (DNA) sequence [1].

Epigenetics is now defined as modifications of DNA or factors associated with DNA, carrying an information that can be heritable but without inducing any changes into the DNA sequence.

These chromatin modifications are well described and summarized in many reviews [2-5].

They essentially include:

i) Methylation of DNA in particular; 5-methylcytosine (5mC) enriched CpG islands: This pattern, especially when found at gene promoters, is associated in most cases to inhibition of gene expression. DNA methylation is catalyzed by DNA methyltransferases (DNMT), including DNMT3A/3B that mediates the *de novo* methylation and DNMT1 that mediate maintenance of DNA methylation on the newly synthesized DNA strand during DNA replication [6].

ii) Chromatin modifications, based on the post-translational regulation of histones tails: They can ultimately modulate the compaction of the chromatin and can be associated with gene expression or repression [7]. Among histone tail modifications, acetylation and methylation of histones are probably the most studied histone marks. Histone acetylation is associated with a decompaction of the chromatin and an increased accessibility of gene promoters and enhancers, allowing transcription factor binding and regulation of gene expression [8]. Chromatin acetylation is carried out by histone acetyltransferases (HATs) and deacetylation by histone deacetyltransferases (HDACs). Histone methylations could be associated with both activation (H3K4me3, H3K36me3) or repression (H3K9me3, H3K27me3) of gene expression and is regulated by histone methyltransferases (HMTs or KMTs), adding 1 to 3 methyl groups on histone lysine or arginine. These modifications can be reversed by histones demethylases (HDMs or KDMs) that specifically recognize mono, di, or tri-methyl marks [9].

iii) Non-coding ribonucleic acid (RNA), including micro RNA (miRNA) and long non-coding RNA (lncRNA): miRNAs are small RNA molecules, acting at post-transcriptional level to block translation by degrading messenger RNA (mRNA) [10]. lncRNA regulate gene expression by regulating chromatin structure, mRNA stability, splicing, and post-translational regulation processes [11].

Altogether, these epigenetic mechanisms are involved in many biological processes, such as development, gene imprinting, X inactivation, cell fate decision and cell identity control [9,12-14]. They are also shown to be deregulated in many diseases including cancer. Nonetheless, epigenetic deregulation in cancer is not restricted to cancer cells, but can also be observed in non-malignant cells of the tumor-microenvironment (TME), suggesting that tumor niche could also be targeted by epigenetic therapy [15-18]. Through this review, we have presented an exhaustive account of the epigenetic deregulations observed in the TME, especially of the stromal cell population corresponding to the cancer-associated fibroblasts (CAFs), the suppressive immune cells namely; the regulatory T cells (Tregs) and the myeloid-derived suppressor cells (MDSCs). Finally, we have deliberated the potential impact of epigenetic therapies on the immunosuppressive properties of TME.

Genetic and epigenetic deregulation in cancer cells

Cancer is a genetic and epigenetic disease, where genetic alterations and epigenetic deregulations are entangled from the beginning to the end of the oncogenic process [19]. Genetic alterations in cancer cells can occur in both oncogenes and tumor suppressors genes (TSGs). Interestingly, some additional mutations are not oncogenic *per se* but favor the crosstalk with TME thus linking tumor genetics and tumor niche features, as observed in follicular lymphoma (FL) [20]. In addition, in many cancers, genetic alterations can occur through gene coding for epigenetic regulators. As an example, mutations in genes with a role of catalyzing the post-translational modification of histones, such as the histone H3 lysine 4 (H3K4) methyltransferases KMT2D and KTM2C, the histone acetyltransferases CREBBP and EP300, and the histone H3 lysine 27 (H3K27) methyltransferase EZH2, are a hallmark of FL [21]. About 70% of FL patients harbor at least 2 mutations in chromatin-modifying genes, making the targeting of epigenetic modifiers an attractive therapeutic target in this disease. Whilst, the majority of EZH2 mutations are subclonal events, mutations of CREBBP probably arise as early driver genetic events residing within tumor cell progenitors [22]. It is also the case for *de novo* acute myeloid leukemia (AML) where genetic alteration of epigenetic regulators (DNMT3A and ten-eleven-translocation 2 (TET2)) can be observed at the beginning of the oncogenic process [23]. Besides mutations, epigenetic deregulations are observed in virtually all cancer types, with disruptions of DNA methylation, histone modifications and non-coding RNAs mechanisms and are already extensively described [24,25]. Interestingly like

genetic alterations, epigenetic deregulations in cancer cells could also impact the establishment of a supportive TME. As an example, overexpression of the H3K27 demethylase KDM6B impacts the regulation of the NF- κ B pathway in melanoma cancer, with the overexpression of stanniocalcin 1 (STC1) and chemokine (C-C motif) ligand 2 (CCL2) by melanoma tumor cells, leading simultaneously to macrophage infiltration, angiogenesis, and lung metastases [26].

Tumor microenvironment leads tumor progression

Tumor cells live in a complex ecosystem formed by infiltrating immune cells, endothelial cells, and stromal cells [27]. There is increasing evidence suggesting the involvement of TME in many tumorigenic processes including tumor cell proliferation and survival, immune escape, metastatic process, angiogenesis, and resistance to therapies [28-30]. As an example, in GC-derived B-cell lymphomas, neutrophils recruited through production of IL-8 by stromal cells, could provide supportive effect to FL B-cells *in-vitro* [31]. In addition, tumor associated macrophages (TAM), could also lead to tumor progression through the establishment of an immune-suppressive microenvironment notably via the production of chemokines like CCL17, CCL18 and CCL22, with the consequence to sequester Tregs and inhibits immune responses [32-34]. Immune suppression in the TME is also mediated by stromal cells such as CAFs and myeloid cells like MDSCs and will be further elaborated in the next sections. Interestingly, the emergence of immune-checkpoint therapy has revealed a major role of TME in the resistance to immune-checkpoint inhibitors. In particular through physical blockade of access by immune cells to tumor bed (immune-excluded tumors) and inhibition of immune cell activation/cytotoxicity (inflamed tumors), revealing a key role of immune cell number, localization, and activation in patient clinical outcome [35,36]. Recently, there have been scientific studies that describe the potential for combining epigenetic inhibitors with immune checkpoint inhibitors, as a mechanism of cancer therapy [37-39], This emphasizes the desideratum to satisfactorily discern the role of epigenetic deregulations in the emergence of immunomodulating cellular properties of the TME as well as the impact of epigenetic therapies on these cells.

II) Epigenetic deregulation mechanisms in CAFs

Stromal cell compartment

Cancer-associated fibroblasts (CAFs), represents one of the most abundant stromal cell populations in the tumor microenvironment. They are frequently described as myofibroblast characterized by the expression of α -sma and have been shown to enhance tumor phenotypes, with critical role in tumor initiation, progression, dissemination, immune escape, and drug resistance [40]. Based on these observations, CAFs emerged as a key player in the TME with numerous reports describing the strong pro-tumorigenic properties of CAFs [41]. These pro-tumorigenic properties are mediated as an example, by the expression of stromal cell-derived factor 1 (SDF-1), also called CXCL12, which could lead to the recruitment and activation of Tregs [42,43]. CAFs also create an immunosuppressive microenvironment through the production of TGF β and the establishment of a particular extra-cellular matrix (ECM) environment that blocks immune cell trafficking associated with immune-checkpoint therapy failure [35,44]. To date, it is quite difficult to study native CAF subpopulations due to their low numbers and lack of characterization for specific surface markers. These difficulties in describing common CAF markers can be ascribed to their multiple putative origins, including mesenchymal stromal cells, activation of resident fibroblasts, pericytes and epithelial cells that follow epithelial-to-mesenchymal transition (EMT), or conversion of endothelial cells [45]. Moreover, a recent study elegantly demonstrates that CAFs are a heterogeneous population *in situ* with different subsets of CAFs carrying specific phenotypes, gene expression profiles, and functions in a given TME [42].

Another layer of complexity could come from the fact that not only are tumors spatially heterogeneous, but CAFs themselves are a heterogeneous population, with different phenotypes and functions depending on their localization, as well as their origins [46]. In particular; solid tumors frequently include tertiary lymphoid structures, that contain CAFs with features of lymphoid stromal cells that regulate immune cell recruitment and activation within lymphoid organs. Presence of these tertiary lymphoid structures are at least in colon cancer, associated with a better outcome [47], suggesting possible anti-tumoral activities [48]. Conversely, infiltration by draining lymph nodes of tumor cells was shown to trigger reprogramming of resident lymphoid stromal cells into CAFs leading to a reduced capacity to promote leukocyte recruitment, migration, and activation; a functional phenotype associated

with a pro-tumoral activity [49]. Similar observation was made in FL where the primary tumor site is the lymph node and where infiltrating lymphoid stromal cells present a specific phenotype. These cells notably overexpress CXCL12 under the influence of T-cell-derived IL-4 and trigger FL B cell activation, migration, and adhesion [50]. However, CAFs origin in tumor-invaded lymph node is still a matter of debate and is not fully elucidated. Interestingly, bone marrow is also penetrated in about 70% of FL patients and CXCL12 is also overexpressed in FL bone marrow stromal cells. Moreover, bone marrow stromal cells also overexpress CCL2 and IL-8 that contributes to the recruitment and activation of tumor-supportive monocyte and neutrophils [31,51].

As discussed above, CAFs are heterogeneous, arising from different cells of origin and displaying both pro and anti-tumoral activities. To date, there is no clear report showing that genetic alterations could be the drivers of CAF phenotype [45]. Conversely, studies showing that CAFs in culture retain some of their properties; suggesting that epigenetic mechanisms could be involved in changes occurring in their transcriptional and phenotypic profiles [15,49].

DNA methylation

It was recently shown that CAFs could be involved in the epigenetic reprogramming of cancer cells, as observed in breast cancer [52]. Moreover, it was proposed that CAFs could present the same DNA methylation pattern as observed in tumor cells, including a global DNA hypomethylation and a local DNA hypermethylation. Such profiles have been observed in CAFs from various cancer types such as gastric cancers [53], colorectal cancer [54], lung cancer [55]. However, these previous results are not in agreement with a recent study in prostate cancer [18]. In this study, the authors by using whole-genome bisulfite sequencing, showed that CAFs from prostate cancer did not exhibit global hypomethylation but rather changed (both increased and decrease) at discrete loci, suggesting that DNA global hypomethylation in CAFs is probably, dependent of the cancer type and should be assessed more carefully with resolutive techniques. These recent observations concerning the whole genome DNA methylation profile of CAFs, both highlights the importance of analyzing DNA methylation for reprogramming of CAFs [56]. Interestingly, local hypermethylation in CAFs was shown to be involved in the conversion of normal fibroblasts into pro-invasive fibroblasts in several cancers (head and neck, lung, and breast cancer). This conversion was mediated through an increased expression of DNMT3b and a local hypermethylation of SHP-1 [57]. In this context, DNMT3b

overexpression was induced by the activation of the JAK1/JAK3 (Janus kinase) signaling pathway by P300 histone acetylation in response to the proinflammatory cytokine leukemia inhibitory factor (LIF), secreted by tumor cells [57]. In addition, inhibition of the RAS inhibitor RASAL1 by DNMT1 was observed in CAFs from renal cancer [58], whereas in prostate cancer aberrant DNA methylation targets the Ras inhibitor RASAL3 [59]. Both lead to the oncogenic activation of Ras and to the modification of CAF metabolism (glutamine synthesis) which are associated with an increase in cancer cell survival and proliferation. DNMT1 was also shown to be up-regulated in breast cancer and its up-regulation is critical for the conversion of normal fibroblasts into CAFs [60]. This conversion was mediated by HuR protein which stabilizes DNMT1 mRNA leading to enhanced pro-inflammatory properties of CAFs via an increased expression of CXCL12, TGF β , and IL-6 [60].

Histone modifications and chromatin remodeling

Histone marks were shown to be involved in the regulation of the functional properties of CAFs. These regulations can be separated in three different observations:

i) Change in histone modifying enzyme gene expression among CAFs. Indeed, it was described in breast cancer that CAFs can overexpress HDAC6, leading to the activation of prostaglandin E2/cyclooxygenase-2 (PGE2/COX2) expression in association with signal transducer and activator of transcription 3 (STAT3) and enhancing the recruitment of MDSCs and Tregs cells [61]. These observations accent that CAF epigenetic deregulations could impact not only cancer cells but also other cells of the TME [62]. HDAC1/3/8 were also shown to be involved in CAF differentiation upon TGF β exposure, increasing tumor growth and ECM secretion [63]. Interestingly, the use of an inhibitor of colony stimulating factor-1 receptor (CSF-1R); the JNJ-40346527, to target the tumor-associated macrophage, was shown to have a pro-tumoral effect mediated by CAFs. Indeed this paradoxical pro-tumoral effect relies on the blockade of the CSF-1-dependant recruitment of HDAC2 to the promoter of granulocyte-specific chemokines in CSF1R-expressing CAF, thus increasing the release of MDSC-recruiting chemokines, that ultimately leads to an increase in polymorphonuclear MDSC (PMN-MDSC) infiltration, ultimately, explaining the limited clinical efficacy of CSF1-R inhibitor [64].

ii) Chromatin remodeling in CAFs. The transcriptional repressor ATF3 and CSL were shown to control CAF activation and are repressed in CAFs. Re-expression of these factors triggers chromatin remodeling and suppression of CAF tumor-promoting properties in mouse models

[65]. In gastric cancer, it was recently shown that CAFs have a distinct H3K27me3 profile compared to normal fibroblasts. This loss was mostly observed with genes involved in stem cell niche, cell growth and tissue development like WNT5A, GREM1, NOG and IGF2 [66]. In addition, the chromatin remodeler HMGA2 enhances the tumor supportive properties of stromal cells in mouse prostate cancer [67].

iii) CAFs can also affect epigenetic landscape in cancer cells. In ovarian cancer, CAFs induce an overexpression of EZH2 leading to an increase of cancer cell migration [68]. Interestingly, epigenetic changes induced by fibroblasts on cancer cells could also be associated with antitumor properties. In particular, it has been described that normal fibroblasts could inhibit breast cancer cell proliferation, through mechanosensitive downregulation and nuclear exit of the H3K9 demethylase JMJD1a leading to a downregulation of YAP/TAZ expression [69]. This observation paves the way to the idea that reversing and inhibiting epigenetic mechanisms involved in CAFs conversion/differentiation could promote generation of CAFs that carry anti-tumor properties.

Non-coding RNA

Many studies highlight the importance of miRNA regulation in CAF pro-tumoral properties [70]. As an example, in prostate cancer, miR-15a and miR-16 are downregulated in CAFs thus reducing the post-transcriptional repression of fibroblast growth factor 2 (Fgf-2) and Fgfr1 and enhancing cancer cell survival, proliferation, and invasiveness [71]. It was also shown that upregulation of miR-409 in CAFs from prostate cancer is sufficient for the differentiation of normal stroma into CAFs [72]. Interestingly, miR-409 can then be released by CAFs via extracellular vesicles and result in enhanced tumor progression and EMT. In ovarian cancer, miR-200 supports the up-regulation of CXCL12 beta isoform expression in a specific CAF subtype and is associated with immunosuppressive cell recruitment [42]. However, in non-mesenchymal ovarian tumors, another subset of CAFs are present which express a miRNA cluster that represses CXCL12 expression, miR141/200c and decreases Tregs recruitment in tumor niche [73], highlighting the bivalent role of CAFs and miRNA regulation.

III) Epigenetic deregulation of the suppressive immune cell subsets

Besides CAFs, the TME also include several pro-tumoral and anti-tumoral immune cell subsets, and epigenetic deregulation of the immune cells in cancer is already described by others [16,62]. However, the crosstalk between CAFs and the main suppressive immune cells, namely Tregs and MDSCs, plays a key role in organizing tumor-promoting microenvironment, highlighting the need to have a holistic comprehension on the epigenetic deregulation of the suppressive cells found in TME. Moreover, understanding epigenetic mechanisms sustaining the development of this immunosuppressive network would be useful in predicting the impact of epigenetics drugs on the TME (Figure 1).

Immune cells in the tumor microenvironment

Many immune cell subsets could be identified within TME and participate in the establishment of an immunosuppressive microenvironment [62]. Among them, Tregs secrete immunosuppressive cytokines like IL-10, IL-35 and tumor growth factor beta (TGF β) [74] and block activation of effector T cells. This blockade is partially mediated through cytotoxic T lymphocyte associated protein 4 (CTLA-4) expression and consumption of IL-2, limiting IL-2 availability [74]. MDSCs are another heterogeneous subset of immune cells also found in TME of many tumors [75]. MDSCs belong to the monocytic (M-MDSC) or granulocytic (PMN-MDSC) lineages and display strong immunosuppressive functions mediated through diverse mechanisms including production of nitric oxide (NO), reactive oxygen species (ROS), immunosuppressive enzymes including arginase 1 (ARG1) or indoleamine 2,3-dioxygenase (IDO), and immunosuppressive cytokines like IL-10 and TGF β , that will altogether impact T cells and naturel Killer (NK) functions [76]. As observed on Tregs, MDSCs immunosuppressive properties are also mediated by expression of the inhibitory immune checkpoint PD-L1 [77].

DNA methylation

DNA methylation was shown to be involved in the regulation of Tregs, the transcription factor forkhead box P3 (FOXP3) is crucial for the development and function of Tregs and its expression is strongly dependent on the Treg-specific demethylated region (TSDR), an epigenetic marker for natural Tregs (nTregs) [78]. DNA demethylation agents used in cancer cell therapy could thus impact Treg population. As it was shown for CAFs and cancer cells,

MDSCs also present a global DNA hypomethylation profile with a local gain of DNA hypermethylation. These are notably shown in ovarian cancer, where MDSCs presents a global DNA hypomethylation profile compared to dendritic cells, with a specific gain of DNA methylation and repression of genes associated with an immunogenic phenotype like S1PR4, RUNX1 and FAS. This loci specific methylation, could be related to an increase of DNMT3A (a *de novo* DNA methyltransferase) expression in MDSC [79]. This neoteric observation seems in contradiction with other studies shown in mice models where Cannabinoid (Δ^9 -Tetrahydrocannabinol) could induce a global hypomethylation of MDSCs and a decrease of DNMT3A and DNMT3B expression by DNA methylation of their promotor. This leads to a higher expression of Arg1 and STAT3, that ultimately promote MDSCs immunosuppressive properties [80,81], suggesting that a common epigenetic enzyme deregulation could have different ramifications on acquisition of tumor supportive properties.

Histone modifications

As observed in CAFs, histone modifications are also found altered in immune cells of tumor niche. As an example, HDAC11 was shown to be a negative regulator of MDSCs expansion in mice, in addition the same study described that MDSC isolated from HDAC11-KO tumor-bearing mice were more suppressive than MDSC, purified from the wild type mice [82]. HDAC11 in co-operation with HDAC6 was also shown to be involved in the regulation of IL-10 expression (a cytokine known to recruit immune-suppressive cells like MDSCs) by antigen presenting cells (APC). This suggested the involvement of this mechanism during tumorigenesis to promote an immunosuppressive TME. Besides HDAC11, HDAC2 was shown to be involved in the conversion of M-MDSCs to PMN-MDSCs with higher pro-tumoral properties through the inhibition of the retinoblastoma gene 1 (RB1) [83]. Moreover, the H3K4 methyltransferase SETD1B activates nitric oxide synthase 2 (nos2) expression in MDSCs, leading to an inhibition of T cell-activation, and is associated with an anti-tumor immune response [84].

Epigenetic deregulation in tumor cells can also indirectly lead to an increase of the immunosuppressive properties of TME. In ovarian cancers, EZH2 and DNMT1 are involved in gene repression of T helper 1 chemokines CXCL9 and CXCL10, associated with a decrease in CD8⁺ effector T cell infiltration [85]. In colon cancer, T cell recruitment is also impacted by the

epigenetic regulation of CXCL9 and CXCL10 expression by H3K27me3 repression marks which is modulated by an EZH2/KDM6B balance [86].

Non-coding RNA

As observed for CAFs, it is well described that miRNA are involved in MDSCs identity and acquisition of their pro-tumoral properties [16,76]. Exosomes from glioma cells produced in hypoxic condition induce MDSCs activation by transferring miR-29a and miR-92a. These miRs enhance the proliferation and function of MDSCs by targeting HMG-box transcription factor 1 (Hbp1), a mitosis inhibitor protein and protein kinase CAMP-dependent type 1 regulatory subunit alpha (Prkar1 α), an inhibitor of the STAT3 pathway activation [87]. In glioma as well, miR-10a and miR-21 that target RAR-related orphan receptor alpha (RORA) and phosphatase and tensin homolog (PTEN) respectively are also transmitted to MDSCs via exosomes from cancer cells, leading to an enhanced MDSCs differentiation and activation [88]. Moreover, in melanoma, a set of miRs (miR-146a, miR-155, miR-125b, miR-100, let-7e, miR-125a, miR-146b, miR-99b) have been associated with MDSC differentiation and poor clinical response to immune checkpoint therapy [89]. Interestingly, MDSCs in epithelial ovarian cancer can also influence TME polarization and function through the transmission of miR-21 and miR-29a to T cells, leading to the blockade of STAT3 signaling pathway and increase in immunosuppressive Tregs [90].

IV) Epigenetic therapies

Epigenetic deregulations in tumor cells relies on epigenetic enzymes that can be targeted by epigenetic drugs; many epigenetic drugs (epi-drugs) have been developed, essentially targeting the DNA-methylation machinery and HDACs [91]. The development of epi-drugs is still an intense area of research [4,5,92]. We will not discuss here the direct impacts of these epi-drugs on cancer cells but how these epi-drugs could directly and indirectly impact cells present in the TME (Table1).

TME mediated anti-tumoral impacts of epi-drugs

-DNA methyltransferase inhibitors

DNA demethylating agents (DNMTi), such as 5-aza-2'-deoxycytidine (5-Aza-Cdr), can be used to inhibit DNMTs and are currently approved for the treatment of myelodysplastic syndromes (MDS) and AML [93]. These DNMTi through modulations of the epigenome of the cancer cells could indirectly impact the TME of the cancer cells. As an example, DNMTi could mimic a viral infection in cancer cells called "viral mimicry", [94,95] through the re-expression of endogenous retrovirus in cancer cells and formation of double stranded RNA. This "viral mimicry" by creating an inflammatory context, favor the activation and recruitment of T lymphocytes and in consequence, lead to an increased efficacy of immunotherapy strategies [95,96]. Interestingly, DNMTi can also impact directly the TME. In particular, it was recently proposed that DNMTi could prevent CD8+ T cell exhaustion, by allowing them to retain their effectors functions [97]. Moreover, in a mice model of breast cancer, It was shown that DNMTi could impact MDSCs by reducing their expansion and potentially diminishing their immunosuppressive properties, thus favoring adoptive T cell transfer [98]. This observation confirmed previous results describing a direct impact of 5-Aza-CdR on MDSCs proliferation, in mice models of prostate cancer adenocarcinoma and lung cancer [99]. In addition, it was shown that DNMT3a genetic inhibition, is sufficient to suppress MDSCs immunosuppressive properties abrogating their capacity to suppress CD8+ T cell proliferation and the production of IFN γ in the context of ovarian cancer [79]. Finally, to date, only one study describes the impact of DNMTi on CAF. In this study, done on human fibroblasts from various cancers (head and neck, breast, lung) the author described a constitutive activation of the JAK1/STAT3 pathway, involving both the de novo methyltransferase (DNMT3b) and DNMT1 for the maintenance of DNA methylation to stably repress the PTPN6 tyrosine phosphatase leading to the acquisition of the pro-tumoral properties of CAFs [57]. These pro-tumoral properties could be then reversed by DNMTi treatment in combination with JAK1/2 inhibitors.

-Histone modifying enzyme inhibitors

Only few studies, have established the impacts of histone modifying enzyme on CAFs. One study describes the role of Scriptaid ,a selective inhibitor of HDACs, 1, 3 and 8, on CAFs, in an *in-vitro* and *in-vivo* model of melanoma [63]. They observed, that blocking of the HDACs (1,3 and 8) supresses the activation of the TGFbeta pathway in CAFs leading to a reduction of the

CAFs tumor supportive properties. In a PDX model of pancreatic ductal adenocarcinoma (PDAC), JQ1, an inhibitor of chromatin readers which contain bromo- and extra-terminal domain (BET) can reverse CAF phenotype by downregulating key pathways involved in CAF activation, in particular the activation of the TGF β pathway [100]. These two studies highlight the importance of blocking the TGFbeta pathway on CAFs, a pathway that was recently shown to be associated with the immune-suppressive properties of CAFs [44]. In addition, a recent study, described that methyltransferase inhibitors (targeting both histones and DNA methylation), could block the capacity of CAFs to remodel the ECM and prevent metastasis formation in a model of breast cancer, through the interaction of SNAIL with the methyltransferase PRMT1 and PRMT4 [101].

To finish, indirect impact of histone modifying enzyme inhibitors on the TME, mediated by the cancer cells were described. As an example, in murine ovarian cancer, 5-Aza-CdR induces a type I IFN response, leading to the activation of cytotoxic T cells and NK cells and reducing the percentage of MDSCs. These anti-tumor effects are enhanced by a combination of HDACi and immune checkpoint inhibitors [102]. EZH2 inhibitors were also shown to inhibit Tregs, and to improve anti-CTLA-4 therapy, highlighting our incomplete knowledge of the impact of epi-drugs on anti-tumor immune response [103]. Finally, Immune checkpoint inhibitors in association with the two HDACi, entinostat or mocetinostat, decrease MDSCs recruitment and increase CD8+ T cell infiltrations in TME of breast, pancreatic cancer and non-small cell lung cancer [104,105].

Epi-drugs promote a pro-tumoral microenvironment

However, epi-drugs could have a dual effect on the stromal compartments. Indeed, DNMTi were also shown to induce immunosuppressive MSC through upregulation of COX2 [106], an effect that could be beneficial in immune diseases where immune cells need to be tempered, but could have inverse effects in cancer. Moreover, DNMTi also increases the immunosuppressive properties of MDSCs through STAT3 and ARG1 activation [80]. the EZH2 inhibitor GSK126 can induce an increase of MDSCs while CD4 + and CD8+ T cells are decreased [107]. In addition, some studies suggest that HDACi could enhance MDSCs proliferation [108,109] as well as Treg differentiation [110-112]. In addition, in PDAC, HDACi were shown to induce a supportive stroma and inhibition of HDAC2 in CAFs leading to an increased secretion of tumor-supportive cytokines and chemokines [113]. These were also confirmed in

breast tumors, where HDAC1 inhibition in CAFs leads to an increased expression of osteopontin and promote tumor growth [114]. These observations highlight that epi-drugs could have a dual impact on TME, that need to be cautiously analyzed by further researches.

V) Future perspectives

In conclusion, epigenetic deregulation in TME, especially in CAFs and MDSCs, are involved in the establishment of an immunosuppressive microenvironment. Moreover, epigenetic therapies targeting cancer cells, such as DNMTi and HDACi could favor or repress the tumor-supportive activities of TME. To date, there is a scarcity of studies addressing the direct impact of these epigenetic therapies on cells present in the TME. As discoveries in the role of TME towards tumor progression and resistance to therapy advances, it will become inevitable to address how these treatments could impact cells of the TME. Especially, one challenge that surfaces, would be the specific targeting of the TME to deliver epigenetic drugs. Development of strategies to deliver epigenetic drugs is an intense area of research [115,116]. Targeting of the TME is currently being developed and achievements thus far, in this field of research have been epitomized in this review [116]. However, identification of the right ligand to target specifically CAFs will be a prerequisite and is still a matter of research [116]. In addition, with the development of the single-cell epigenomics technique [117-119], it will be possible to have a better understanding of the role of epigenetic modifications for the fine-tuning of TME differentiation and functions. Such techniques, will be a prerequisite for a better design of adequate epigenetic therapeutic strategies.

Executive summary

Epigenetic mechanisms deregulation in CAFs

- Cancer associated fibroblasts (CAFs) are key players of the tumor microenvironment (TME) and lead to tumor progression.
- CAFs differ from normal fibroblasts, in contrary to cancers cells. CAFs do not present genomic alterations, highlighting others mechanisms involved in their modification and associated with their pro-tumorigenic properties.
- CAFs present epigenetic deregulation, like global DNA hypomethylation and local DNA hypermethylation. DNA methylation seems to be critical for CAF conversions. In addition, histone modifications and chromatin remodeling are also observed in CAFs and support their pro-tumorigenic properties.

Epigenetic deregulation of the suppressive immune compartment

- TME include beside CAFs, others immunosuppressive cells and especially regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).
- Immunosuppressive properties of Tregs is mediated in part by DNA demethylation of the Treg-specific demethylated region. In addition, as observed in CAFs and Cancer cells, MDSCs present a global DNA-methylation profile and a local hypermethylation.
- Epigenetic changes observed in MDSCs also include histone modification and Non-coding RNA that are both involved in the acquisition of their immunosuppressive properties.

Epigenetic therapies

- Epigenetic drugs (epi-drugs) were first developed to target cancer cells. However, these epi-drugs can impact directly and indirectly on cells present in the TME
- Epi-drugs could have a dual impact on the immunosuppressive properties of cells present in TME. These Epi-drugs can both favor or repress the tumor-supportive activities of TME
- In future, a better understanding of the role of epigenetic modification in the fine-tuning of TME is a prerequisite for a better design of adequate epigenetic therapeutic strategies.

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451 **Conflicts of interest:** The authors declare no conflicts of interest.

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Revised manuscript

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Treatments	Effects	References
DNA methyltransferase inhibitors	- Activation of “viral mimicry” mechanism - Enhances tumor antigens, reverse CAFs phenotype - Up regulation of cytokines in CD8 T cells - Reactivation of CXCL9/10 chemokine in tumor cells	[57,85,94,95]
Histone methyltransferase inhibitors	- De-repress CXCL9/10 and effector T cells trafficking - Increases immune checkpoint therapy	[85,86,103]
Histone deacetylase inhibitors	- Decreases of MDSCs and increase CD8+ T cells infiltration in association of immune checkpoint inhibitor - Reverses CAFs phenotype - Enhances tumor antigen expression	[63,102,104,105]
Chromatin reader inhibitors	- Reverses CAFs phenotype	[100]

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Table 1. Epigenetic drugs impact both tumor and tumor microenvironment

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Figure 1: Impact of epigenetic regulation on crosstalk between tumor cells and CAFs, MDSCs and T cells of the microenvironment. Immunosuppressive properties and tumor supportive effects of microenvironment cells are under the control of epigenetic mechanisms that are deregulated. These deregulations impact: cytokines, chemokines and exosomes secretion. Altogether they favor tumor progression and metastasis, escape to the immune system and resistance to immune checkpoint therapy. Green box contains the up-regulated epigenetic factors and red box the down-regulated.

Figure 1