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

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Revised manuscript

1 **Abstract**

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

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

20 **I) Introduction**

21 **Epigenetic mechanisms**

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

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

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

28 They essentially include:

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

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

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

51 Altogether, these epigenetic mechanisms are involved in many biological processes, such as
52 development, gene imprinting, X inactivation, cell fate decision and cell identity control [9,12-
53 14]. They are also shown to be deregulated in many diseases including cancer. Nonetheless,
54 epigenetic deregulation in cancer is not restricted to cancer cells, but can also be observed in
55 non-malignant cells of the tumor-microenvironment (TME), suggesting that tumor niche could
56 also be targeted by epigenetic therapy [15-18]. Through this review, we have presented an
57 exhaustive account of the epigenetic deregulations observed in the TME, especially of the
58 stromal cell population corresponding to the cancer-associated fibroblasts (CAFs), the
59 suppressive immune cells namely; the regulatory T cells (Tregs) and the myeloid-derived
60 suppressor cells (MDSCs). Finally, we have deliberated the potential impact of epigenetic
61 therapies on the immunosuppressive properties of TME.

62 63 **Genetic and epigenetic deregulation in cancer cells**

64 Cancer is a genetic and epigenetic disease, where genetic alterations and epigenetic
65 deregulations are entangled from the beginning to the end of the oncogenic process [19].
66 Genetic alterations in cancer cells can occur in both oncogenes and tumor suppressors genes
67 (TSGs). Interestingly, some additional mutations are not oncogenic *per se* but favor the
68 crosstalk with TME thus linking tumor genetics and tumor niche features, as observed in
69 follicular lymphoma (FL) [20]. In addition, in many cancers, genetic alterations can occur
70 through gene coding for epigenetic regulators. As an example, mutations in genes with a role
71 of catalyzing the post-translational modification of histones, such as the histone H3 lysine 4
72 (H3K4) methyltransferases KMT2D and KTM2C, the histone acetyltransferases CREBBP and
73 EP300, and the histone H3 lysine 27 (H3K27) methyltransferase EZH2, are a hallmark of FL
74 [21]. About 70% of FL patients harbor at least 2 mutations in chromatin-modifying genes,
75 making the targeting of epigenetic modifiers an attractive therapeutic target in this disease.
76 Whilst, the majority of EZH2 mutations are subclonal events, mutations of CREBBP probably
77 arise as early driver genetic events residing within tumor cell progenitors [22]. It is also the
78 case for *de novo* acute myeloid leukemia (AML) where genetic alteration of epigenetic
79 regulators (DNMT3A and ten-eleven-translocation 2 (TET2)) can be observed at the beginning
80 of the oncogenic process [23]. Besides mutations, epigenetic deregulations are observed in
81 virtually all cancer types, with disruptions of DNA methylation, histone modifications and non-
82 coding RNAs mechanisms and are already extensively described [24,25]. Interestingly like

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

89

90 **Tumor microenvironment leads tumor progression**

91 Tumor cells live in a complex ecosystem formed by infiltrating immune cells, endothelial cells,
92 and stromal cells [27]. There is increasing evidence suggesting the involvement of TME in
93 many tumorigenic processes including tumor cell proliferation and survival, immune escape,
94 metastatic process, angiogenesis, and resistance to therapies [28-30]. As an example, in GC-
95 derived B-cell lymphomas, neutrophils recruited through production of IL-8 by stromal cells,
96 could provide supportive effect to FL B-cells *in-vitro* [31]. In addition, tumor associated
97 macrophages (TAM), could also lead to tumor progression through the establishment of an
98 immune-suppressive microenvironment notably via the production of chemokines like CCL17,
99 CCL18 and CCL22, with the consequence to sequester Tregs and inhibits immune responses
100 [32-34]. Immune suppression in the TME is also mediated by stromal cells such as CAFs and
101 myeloid cells like MDSCs and will be further elaborated in the next sections. Interestingly, the
102 emergence of immune-checkpoint therapy has revealed a major role of TME in the resistance
103 to immune-checkpoint inhibitors. In particular through physical blockade of access by
104 immune cells to tumor bed (immune-excluded tumors) and inhibition of immune cell
105 activation/cytotoxicity (inflamed tumors), revealing a key role of immune cell number,
106 localization, and activation in patient clinical outcome [35,36]. Recently, there have been
107 scientific studies that describe the potential for combining epigenetic inhibitors with immune
108 checkpoint inhibitors, as a mechanism of cancer therapy [37-39], This emphasizes the
109 desideratum to satisfactorily discern the role of epigenetic deregulations in the emergence of
110 immunomodulating cellular properties of the TME as well as the impact of epigenetic
111 therapies on these cells.

112

113

114 **II) Epigenetic deregulation mechanisms in CAFs**

115 **Stromal cell compartment**

116 Cancer-associated fibroblasts (CAFs), represents one of the most abundant stromal cell
117 populations in the tumor microenvironment. They are frequently described as myofibroblast
118 characterized by the expression of α -sma and have been shown to enhance tumor
119 phenotypes, with critical role in tumor initiation, progression, dissemination, immune escape,
120 and drug resistance [40]. Based on these observations, CAFs emerged as a key player in the
121 TME with numerous reports describing the strong pro-tumorigenic properties of CAFs [41].
122 These pro-tumorigenic properties are mediated as an example, by the expression of stromal
123 cell-derived factor 1 (SDF-1), also called CXCL12, which could lead to the recruitment and
124 activation of Tregs [42,43]. CAFs also create an immunosuppressive microenvironment
125 through the production of TGF β and the establishment of a particular extra-cellular matrix
126 (ECM) environment that blocks immune cell trafficking associated with immune-checkpoint
127 therapy failure [35,44]. To date, it is quite difficult to study native CAF subpopulations due to
128 their low numbers and lack of characterization for specific surface markers. These difficulties
129 in describing common CAF markers can be ascribed to their multiple putative origins, including
130 mesenchymal stromal cells, activation of resident fibroblasts, pericytes and epithelial cells
131 that follow epithelial-to-mesenchymal transition (EMT), or conversion of endothelial cells
132 [45]. Moreover, a recent study elegantly demonstrates that CAFs are a heterogeneous
133 population *in situ* with different subsets of CAFs carrying specific phenotypes, gene expression
134 profiles, and functions in a given TME [42].

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

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

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

159

160 **DNA methylation**

161 It was recently shown that CAFs could be involved in the epigenetic reprogramming of cancer
162 cells, as observed in breast cancer [52]. Moreover, it was proposed that CAFs could present
163 the same DNA methylation pattern as observed in tumor cells, including a global DNA
164 hypomethylation and a local DNA hypermethylation. Such profiles have been observed in CAFs
165 from various cancer types such as gastric cancers [53], colorectal cancer [54], lung cancer [55].
166 However, these previous results are not in agreement with a recent study in prostate cancer
167 [18]. In this study, the authors by using whole-genome bisulfite sequencing, showed that CAFs
168 from prostate cancer did not exhibit global hypomethylation but rather changed (both
169 increased and decrease) at discrete loci, suggesting that DNA global hypomethylation in CAFs
170 is probably, dependent of the cancer type and should be assessed more carefully with
171 resolutive techniques. These recent observations concerning the whole genome DNA
172 methylation profile of CAFs, both highlights the importance of analyzing DNA methylation for
173 reprogramming of CAFs [56]. Interestingly, local hypermethylation in CAFs was shown to be
174 involved in the conversion of normal fibroblasts into pro-invasive fibroblasts in several cancers
175 (head and neck, lung, and breast cancer). This conversion was mediated through an increased
176 expression of DNMT3b and a local hypermethylation of SHP-1 [57]. In this context, DNMT3b

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

188

189 **Histone modifications and chromatin remodeling**

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

192 i) Change in histone modifying enzyme gene expression among CAFs. Indeed, it was described
193 in breast cancer that CAFs can overexpress HDAC6, leading to the activation of prostaglandin
194 E2/cyclooxygenase-2 (PGE2/COX2) expression in association with signal transducer and
195 activator of transcription 3 (STAT3) and enhancing the recruitment of MDSCs and Tregs cells
196 [61]. These observations accent that CAF epigenetic deregulations could impact not only
197 cancer cells but also other cells of the TME [62]. HDAC1/3/8 were also shown to be involved
198 in CAF differentiation upon TGF β exposure, increasing tumor growth and ECM secretion [63].
199 Interestingly, the use of an inhibitor of colony stimulating factor-1 receptor (CSF-1R); the JNJ-
200 40346527, to target the tumor-associated macrophage, was shown to have a pro-tumoral
201 effect mediated by CAFs. Indeed this paradoxical pro-tumoral effect relies on the blockade of
202 the CSF-1-dependant recruitment of HDAC2 to the promoter of granulocyte-specific
203 chemokines in CSF1R-expressing CAF, thus increasing the release of MDSC-recruiting
204 chemokines, that ultimately leads to an increase in polymorphonuclear MDSC (PMN-MDSC)
205 infiltration, ultimately, explaining the limited clinical efficacy of CSF1-R inhibitor [64].

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

209 [65]. In gastric cancer, it was recently shown that CAFs have a distinct H3K27me3 profile
210 compared to normal fibroblasts. This loss was mostly observed with genes involved in stem
211 cell niche, cell growth and tissue development like WNT5A, GREM1, NOG and IGF2 [66]. In
212 addition, the chromatin remodeler HMGA2 enhances the tumor supportive properties of
213 stromal cells in mouse prostate cancer [67].

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

223

224 **Non-coding RNA**

225 Many studies highlight the importance of miRNA regulation in CAF pro-tumoral properties
226 [70]. As an example, in prostate cancer, miR-15a and miR-16 are downregulated in CAFs thus
227 reducing the post-transcriptional repression of fibroblast growth factor 2 (Fgf-2) and Fgfr1 and
228 enhancing cancer cell survival, proliferation, and invasiveness [71]. It was also shown that
229 upregulation of miR-409 in CAFs from prostate cancer is sufficient for the differentiation of
230 normal stroma into CAFs [72]. Interestingly, miR-409 can then be released by CAFs via
231 extracellular vesicles and result in enhanced tumor progression and EMT. In ovarian cancer,
232 miR-200 supports the up-regulation of CXCL12 beta isoform expression in a specific CAF
233 subtype and is associated with immunosuppressive cell recruitment [42]. However, in non-
234 mesenchymal ovarian tumors, another subset of CAFs are present which express a miRNA
235 cluster that represses CXCL12 expression, miR141/200c and decreases Tregs recruitment in
236 tumor niche [73], highlighting the bivalent role of CAFs and miRNA regulation.

237

238

239

240 **III) Epigenetic deregulation of the suppressive immune cell subsets**

241 Besides CAFs, the TME also include several pro-tumoral and anti-tumoral immune cell subsets,
242 and epigenetic deregulation of the immune cells in cancer is already described by others
243 [16,62]. However, the crosstalk between CAFs and the main suppressive immune cells, namely
244 Tregs and MDSCs, plays a key role in organizing tumor-promoting microenvironment,
245 highlighting the need to have a holistic comprehension on the epigenetic deregulation of the
246 suppressive cells found in TME. Moreover, understanding epigenetic mechanisms sustaining
247 the development of this immunosuppressive network would be useful in predicting the impact
248 of epigenetics drugs on the TME (Figure 1).

249

250 **Immune cells in the tumor microenvironment**

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

264

265 **DNA methylation**

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

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

283

284 **Histone modifications**

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

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

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

304

305 **Non-coding RNA**

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

321

322 **IV) Epigenetic therapies**

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

329

330

331

332

333 **TME mediated anti-tumoral impacts of epi-drugs**

334 **-DNA methyltransferase inhibitors**

335 DNA demethylating agents (DNMTi), such as 5-aza-2'-deoxycytidine (5-Aza-Cdr), can be used
336 to inhibit DNMTs and are currently approved for the treatment of myelodysplastic syndromes
337 (MDS) and AML [93]. These DNMTi through modulations of the epigenome of the cancer cells
338 could indirectly impact the TME of the cancer cells. As an example, DNMTi could mimic a viral
339 infection in cancer cells called "viral mimicry", [94,95] through the re-expression of
340 endogenous retrovirus in cancer cells and formation of double stranded RNA. This "viral
341 mimicry" by creating an inflammatory context, favor the activation and recruitment of T
342 lymphocytes and in consequence, lead to an increased efficacy of immunotherapy strategies
343 [95,96]. Interestingly, DNMTi can also impact directly the TME. In particular, it was recently
344 proposed that DNMTi could prevent CD8+ T cell exhaustion, by allowing them to retain their
345 effectors functions [97]. Moreover, in a mice model of breast cancer, It was shown that DNMTi
346 could impact MDSCs by reducing their expansion and potentially diminishing their
347 immunosuppressive properties, thus favoring adoptive T cell transfer [98]. This observation
348 confirmed previous results describing a direct impact of 5-Aza-CdR on MDSCs proliferation, in
349 mice models of prostate cancer adenocarcinoma and lung cancer [99]. In addition, it was
350 shown that DNMT3a genetic inhibition, is sufficient to suppress MDSCs immunosuppressive
351 properties abrogating their capacity to suppress CD8+ T cell proliferation and the production
352 of IFN γ in the context of ovarian cancer [79]. Finally, to date, only one study describes the
353 impact of DNMTi on CAF. In this study, done on human fibroblasts from various cancers (head
354 and neck, breast, lung) the author described a constitutive activation of the JAK1/STAT3
355 pathway, involving both the de novo methyltransferase (DNMT3b) and DNMT1 for the
356 maintenance of DNA methylation to stably repress the PTPN6 tyrosine phosphatase leading
357 to the acquisition of the pro-tumoral properties of CAFs [57]. These pro-tumoral properties
358 could be then reversed by DNMTi treatment in combination with JAK1/2 inhibitors.

359

360 **-Histone modifying enzyme inhibitors**

361 Only few studies, have established the impacts of histone modifying enzyme on CAFs. One
362 study describes the role of Scriptaid ,a selective inhibitor of HDACs, 1, 3 and 8, on CAFs, in an
363 *in-vitro* and *in-vivo* model of melanoma [63]. They observed, that blocking of the HDACs (1,3
364 and8) supresses the activation of the TGFbeta pathway in CAFs leading to a reduction of the

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

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

385

386 **Epi-drugs promote a pro-tumoral microenvironment**

387 However, epi-drugs could have a dual effect on the stromal compartments. Indeed, DNMTi
388 were also shown to induce immunosuppressive MSC through upregulation of COX2 [106], an
389 effect that could be beneficial in immune diseases where immune cells need to be tempered,
390 but could have inverse effects in cancer. Moreover, DNMTi also increases the
391 immunosuppressive properties of MDSCs through STAT3 and ARG1 activation [80]. the EZH2
392 inhibitor GSK126 can induce an increase of MDSCs while CD4 + and CD8+ T cells are decreased
393 [107]. In addition, some studies suggest that HDACi could enhance MDSCs proliferation
394 [108,109] as well as Treg differentiation [110-112]. In addition, in PDAC, HDACi were shown
395 to induce a supportive stroma and inhibition of HDAC2 in CAFs leading to an increased
396 secretion of tumor-supportive cytokines and chemokines [113]. These were also confirmed in

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

400

401 **V) Future perspectives**

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

418

419 **Executive summary**

420 **Epigenetic mechanisms deregulation in CAFs**

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

430 **Epigenetic deregulation of the suppressive immune compartment**

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

439 **Epigenetic therapies**

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

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

452

Revised manuscript

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Revised manuscript

Treatments	Effects	References
DNA methyltransferase inhibitors	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - De-repress CXCL9/10 and effector T cells trafficking - Increases immune checkpoint therapy 	[85,86,103]
Histone deacetylase inhibitors	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - Reverses CAFs phenotype 	[100]

781 **Table 1. Epigenetic drugs impact both tumor and tumor microenvironment**

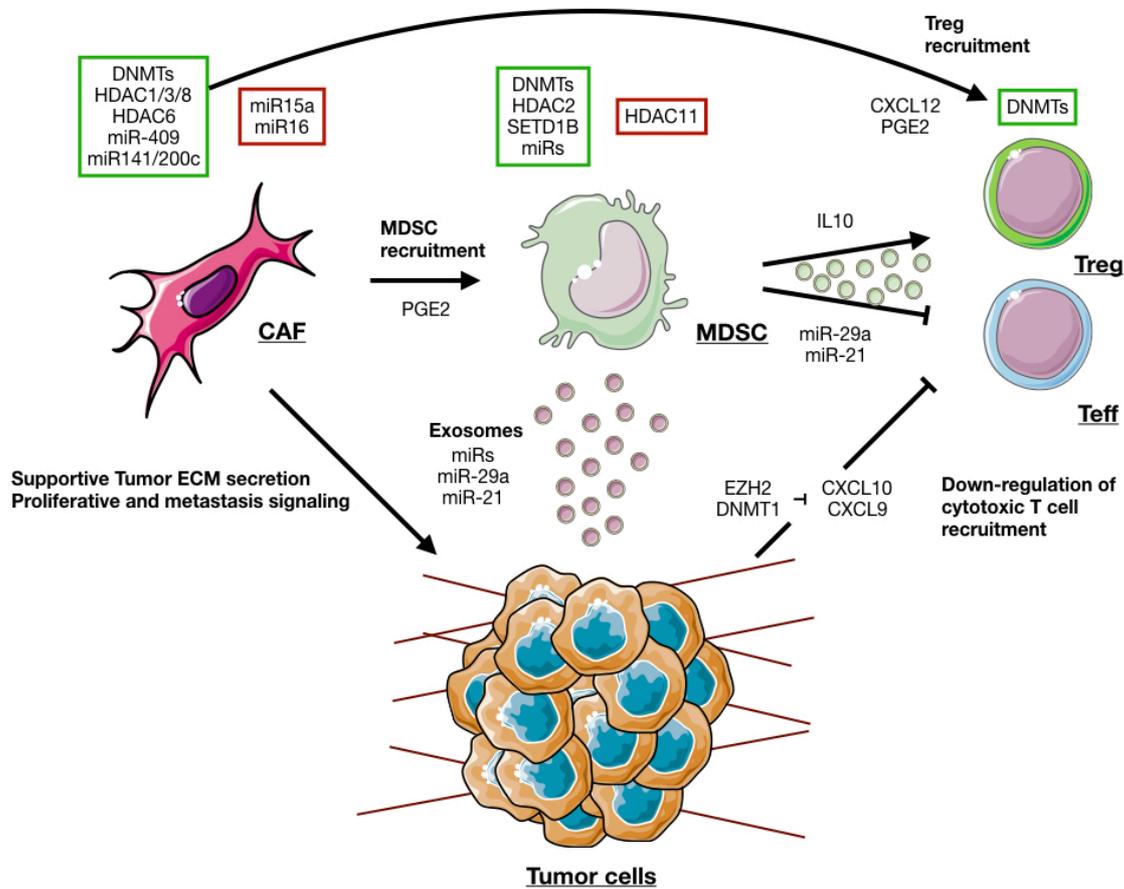
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784 **Figure 1: Impact of epigenetic regulation on crosstalk between tumor cells and CAFs, MDSCs**
 785 **and T cells of the microenvironment.** Immunosuppressive properties and tumor supportive
 786 effects of microenvironment cells are under the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

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Figure 1



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