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Review

The multifaceted effects of metformin on tumor microenvironment

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ABSTRACT

The efficacy of metformin in treating cancer has been extensively investigated since epidemiologic studies associated this anti-diabetic drug with a lower risk of cancer incidence. Since tumors are complex systems, in which cancer cells coexist and interact with several different types of non-malignant cells, it is not surprising that anti-cancer drugs affect not only cancer cells, but also the abundance and functions of cells of the tumor microenvironment. Recent years have seen a wide collection of reports showing how metformin, as well as other complex I inhibitors, may influence cancer progression by modulating the phenotype of non-transformed cells in a tumor. In this review, we particularly focus on the effect of metformin on angiogenesis, cancer-associated fibroblasts, tumor-associated macrophages and cancer immunosuppression.

1. Introduction

Biguanidine metformin is a relatively cheap and well-tolerated drug, traditionally used to lower glycaemia in diabetic patients. It has displayed antitumorigenic effects in both experimental settings and in clinical studies, suggesting beneficial aspects of metformin repurposing for cancer treatment [1,2]. Indeed, ever since epidemiological studies associated metformin treatment with a lower risk of cancer incidence [3], there has been much interest in understanding the mechanisms behind its antitumorigenic effects. Even though several cellular and systemic actions of metformin have been reported [4,5], the most widely accepted explanation is that metformin suppresses tumor progression by inhibiting mitochondrial complex I (CI) [2,6–8]. It must be noted, however, that a binding site for metformin on CI has not been revealed thus far [5]. CI dysfunction, among others, increases cellular AMP/ATP ratio, eventually leading to AMP activated kinase (AMPK) activation, which guards cellular energy homeostasis by slowing down biosynthetic reactions in low nutrient conditions, and its activation has been associated with low proliferative potential [9]. Thus, earlier literature primarily attributed the antitumorigenic effects of metformin to AMPK activity. However, the role of AMPK in cancer is not simply antiproliferative [10], and the latest literature agrees that metformin-mediated CI inhibition challenges tumor progression by leading to aspartate insufficiency [11,12], blocking citrate-dependent de novo lipogenesis [13] and preventing appropriate hypoxic adaptation [14,15]. Despite these phenomena should convincingly result in tumor regression, the assessment of metformin efficacy in treating cancer has provided conflicting data in clinical trials [16]. Different outcomes observed between preclinical and clinical studies may be partly due to dosage effects, since *in vitro* and murine model experiments result in much higher blood concentrations of the drug than what is found in patients [17]. Moreover, a recent study showed that dietary regimen may significantly influence metformin efficacy, as the antitumorigenic effect of the drug was observed in xenograft models only upon fasting-induced hypoglycaemia [18]. Finally, we reason that the tumor response to metformin treatment may also depend on the tumor

Abbreviations: AMPK, AMP activated kinase; Arg1, arginase; BMDM, bone marrow-derived macrophagesn; CAFs, cancer associated fibroblasts; CI, complex I; HIF-1α, hypoxia-inducible factor 1 alpha; LPS, lipopolysaccharide; mTORC1, mammalian target of rapamycin complex I; MDSC, myeloid-derived suppressor cell; NK, natural killer cell; PD-1, Programmed cell death protein 1; PD-L1, programmed death ligand 1; PMA, phorbol myristate acetate; TAM, tumor associated macrophage; TIL, tumor infiltrating lymphocyte; TME, tumor microenvironment; Treg, regulatory T-cells

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Table 1
A list of studies reporting TME-related effects of metformin and/or complex I inhibition.

	TME model	Experimental setting	Effect of metformin (or other CI inhibitors)	Reference
Endothelial cells	indirect effect	MDA-MB-435 (expresses melanocytic markers) xenografts in nude mice	increased VEGFA production in vitro and in vivo, increased microvascular density (reduced Ki-67)	[26]
	indirect effect	A374, Mel-HO, MDA-MB-435 melanoma xenografts	increased VEGFA production in cancer cells	[27]
	indirect effect	H460 lung cancer xenografts in nude mice	BAY 87-2243 CI inhibitor caused HIF-1α deactivation and reduced in HIF1-mediated gene expression	[23]
	indirect effect	HCT116p53-/-	reduced HIF1, VEGF and CAIX in vitro and in vivo in xenografts	[2]
	indirect effect	Human MDA-MB-436 breast cancer in vitro	metformin and phenformin reduced production of angiogenesis-related proteins (IGFBP-2, PDGFAA, VEGF, Angiogenin, MMP-9 and endostatin)	[21]
	in vivo vessels	FVB mice injected with murine MMTV-Erbb2 breast cancer cells	reduced microvascular density and endothelial cell component in the tumors	
	in vivo vessels	matrigel sponge assay in C57Bl6 mice	reduced angiogenesis	[29]
	HUVEC	in vitro	reduced endothelial cell proliferation, migration and network formation	
	indirect effect	HepG2, Huh7 hepatocellular carcinoma cell lines	HIF1, CAIX and Glut1 downregulation in vitro and in vivo in xenografts	[14]
	HUVEC	co-culture with 4T1 or MDA-MB-453 breast cancer cells	reduced proangiogenic capacity of metformin pre-treated cancer cells	[20]
	in vivo vessels (tumor)	4T1 breast cancer tumors in Balb/c mice	reduced vessel leakiness and smaller vessel size/diameter in tumors	
	in vitro	MDA-MB-435 and MDA-MB-231 in vitro	AG311 CI inhibitor destabilized HIF-1α in hypoxia and reduced expression of HIF1-target genes	[24]
	indirect effect	gallbladder xenograft model	HIF1 and VEGF downregulation in gallbladder cancer cells	[19]
	indirect effect	143B (osteosarcoma) and HCT116p53-/- (colorectal cancer) xenografts in nude mice	genetic knock-out of CI in cancer cells prevented HIF-1α stabilization, reduced vessel size and number of pericyte-positive vessels	[22]
Fibroblasts	hTERT-BJ1 (Human immortalized fibroblasts)	co-culture with MDA-MB-231 breast cancer cell lines	overexpression of mitochondrial uncoupling proteins induced high-energy nutrient production in fibroblasts, which may support cancer cell survival in a paracrine fashion	[35]
	MRC5 fibroblast cell line	organotypic co-culture with SKOV3	metformin reduced CAF-mediated support of cancer cell progression	[32]
	MRC5 fibroblast cell line patient-derived CAFs	co-injection of MRC5 and SKOV3 in NOD/SCID mice ovarian cancer	tumors in which MRC5 cells were primed with metformin increased sensitivity to cisplatin reduced IL-6 secretion	
	in vivo CAFs	pancreatic adenocarcinoma 6606PDA orthotopic in C57BL/6	no effect on collagen I deposition by activated stromal cells	[31]
	gastric cancer patient derived CAFs	co-culture with SGC-7901, BGC823, GES-1 and MGT-803 gastric cancer cell lines	suppressed CAF-mediated proclonogenic effect on cancer cells	[34]
	in vivo CAFs	143B (osteosarcoma) and HCT116p53-/- (colorectal cancer) xenografts in nude mice	genetic knock-out of CI in cancer cells associated with stromal component abundance in tumors	[22]

(*continued on next page*)

Table 1 (*continued*)

	TME model	Experimental setting	Effect of metformin (or other CI inhibitors)	Reference
Macrophages	RAW.264.7	in vitro LPS stimulation	inhibited NF-kB pathway (translocation of RALa into the nucleus)	[36]
	RAW.264.7, ex vivo peritoneal macrophages (C57/BL6)	in vitro LPS stimulation	suppressed TNF-α a and IL-6 expression	[37]
	ex vivo PBMCs from healthy human donors	in vitro LPS stimulation, co-culture with BxPC-3 pancreatic cancer	decreased number of CD68+ cells, decreased LPS-induced cytokine secretion (IL-6, IL-8, IL-1b, TNF-α and IL-10)	[38]
	RAW.264.7	in vitro	induced expression of M2 markers (Arg1, IL-4 and IL-10) and downregulated IL-1b and IL-6	[43]
	RAW.264.7	co-culture with HepG2 hepatocellular carcinoma cells	upregulated inflammatory factors (IL-6, IL-1b, TNF-α, MCP-1)	
	in vivo TAMs	pancreatic cancer: PAN02 (in C57BL/6) and AK4.4 (in FVB)	decreased number of TAMs, decreased Arg1 and IL-10 mRNA, decreased IL-1b and CXCL1 protein	[47]
	ex vivo BMDM (C57/BL6)	in vitro LPS stimulation	metformin and rotenone decreased IL-1b, TNF-α and increased IL-10 expression	[40]
	THP-1	in vitro phorbol myristate acetate (PMA) stimulation	reduced CD206 receptor expression, and MRC1 and dectin mRNA	[45]
	RAW.264.7	in vitro IL-13 stimulation	reduced M2 signature (Mrc1, Pparγ, Ccl24, Ccr2, Mgl2, Retnla, and Arg1)	
	in vivo TAMs	Lewis lung carcinoma (in C57BL/6)	no difference in TAM numbers, but reduced CD206 receptor expression	
	THP-1	in vitro PMA stimulation	reduced monocyte to macrophage differentiation (reduced TNF-α, IL-1b, MCP-1)	[39]
	serum cytokine levels	Apo-/- mice (unknown background)	reduced TNF-α, MCP-1 and increased IL-10 in serum	
	THP-1/RAW.264.7	co-culture with LNCap/RM1 prostate cancer	inhibited cancer cell-mediated macrophage migration	[44]
	in vivo TAMs	adenocarcinoma of the mouse prostate/human prostate tumors	reduced macrophage abundance in tumor tissue	
	ex vivo BMDM (C57BL/6)	calvaria model of osteolysis	increased CD206 expression, decreased TNF-α and IL-6, increased IL-10	[41]
	in vivo adipose tissue macrophages	obese C57BL/6 mice	decreased serum levels of the inflammatory cytokines IL-6 and TNF-α, lowered the expression of the M1 macrophage markers CD11c and MCP-1 in adipose tissue	[42]
	RAW.264.7	in vitro palmitate stimulation	reduced the secretion of IL-6 and TNF-α	
	ex vivo BMDM (C57BL/6)	in vitro palmitate stimulation	decreased the ratio of M1/M2 macrophages (increased CD206 expression)	
	in vivo TAMs	4T1 breast cancer in BALB/c	increased CD68+ in tumors, higher iNOS, lower Arg1 expression	[46]
	RAW.264.7	in vitro IL-13 stimulation	reduced Arg1 expression, no effect on iNOS	
	in vivo TAMs	143B (osteosarcoma) and HCT116p53-/- (colorectal cancer) xenografts in nude mice	higher number of F4/80, higher number of Ly6C- monocytes, no difference in CD206 expression	[22]
	in vivo TAMs		genetic knock-out of CI in cancer cells induced higher TAM abundance, higher number of Ly6C- monocytes, no difference in CD206 expression and reduction of Arg1 expression	
T-cells	in vivo TILs	RLmale1 tumors in Balb/c mice	increased total number of TILs	[51]
	ex vivo TILs from RLmale1 tumors	in vitro PMA/ionomycin stimulation	boosted multifunctionality (triple cytokine secretion: IL-2, IFN-γ, TNF-α)	
	in vivo TILs	murine melanoma cells (B16F10) in C57BL/6	increased T-cell infiltration into tumor	[53]
	ex vivo CD4+ cells from murine melanoma tumors (B16F10)	in vitro PMA/ionomycin stimulation	increased CD4+Foxp3+IL-10+ and reduced ROR-γ+IL-17A + CD4+ T-cells	
	indirect effect	B16-F10 (melanoma) and MC38 (colon adenocarcinoma) in C57BL/6	decreased tumor cell oxygen consumption rate, but increased T-cell oxygen consumption rate; higher number of tumor infiltrating activated T-cells	[55]
	in vivo CTL	4T1 breast cancer in BALB/c	decreased PD-L1 levels in cancer cells, increased the CD8+ population	[52]
	in vivo T-cell subsets	Hepa1-6 orthotopic in C57BL/6 (HCC model)	decreased the numbers of Th1 and Th17 cells (lower levels of IL22)	[60]
	in vivo TILs	fibrosarcoma methA, RLmale1, B6 fibrosarcoma MCA and B16 melanoma M05 in BALB/c	decreased the Treg/CD4+ T-cell ratio in tumors	[61]
	ovarian cancer patient derived TILs	co-culture of MDSCs with CD8+ T-cells	decreased enzymatic activity of MDSCs, improved CD8+ T-cell antitumor functions (induced IFN-γ, granzyme B and perforin production)	[56]
	in vivo TILs	human ovarian cancers	increased production of granzyme B and perforin	

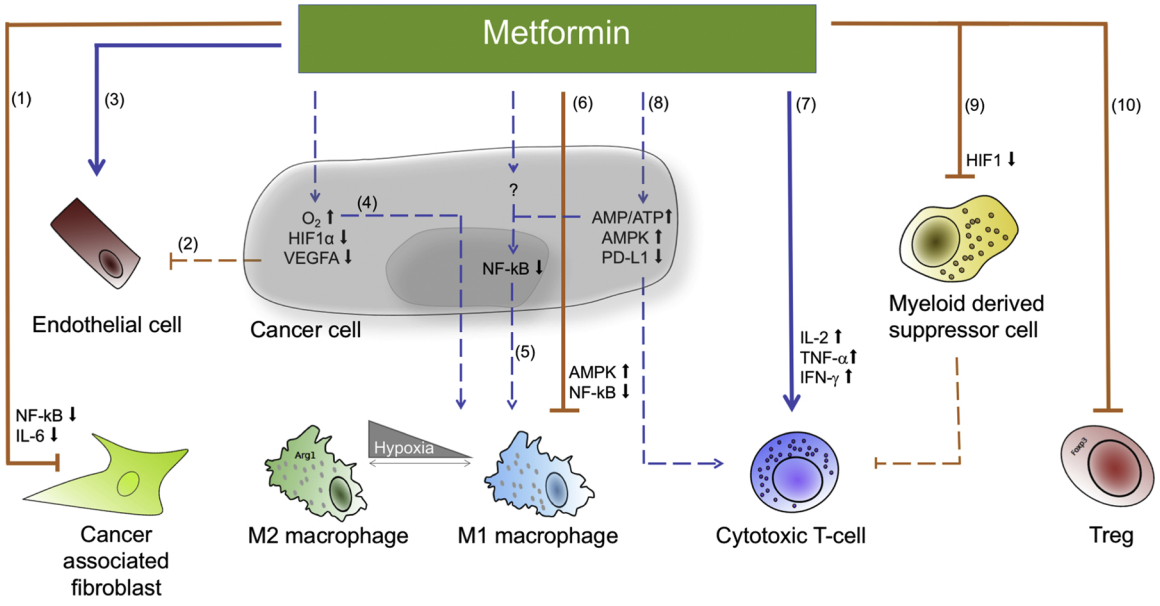

Fig. 1. Tumor microenvironment-related effects of metformin. Metformin was found to compromise tumor progression by directly blocking CAF-derived NF-kB proinflammatory signals (1). In cancer cells, metformin increases intracellular oxygen concentrations, leading to the block of HIF1 signaling and subsequent reduction in cancer cell-derived VEGFA-mediated angiogenesis (2). On the other hand, a direct protective effect of metformin on endothelial cells has been reported (3). Metformin-mediated increase of intracellular oxygen concentrations may contribute also to a general reduction of hypoxia in a tumor, which may shift TAM phenotype towards M1 (4). Furthermore, metformin may lead to NF-kB downregulation in cancer cells, via activation of AMPK or other unknown factors, modifying their inflammatory repertoire and eventually promoting M1 phenotype (5). On the other hand, when used to directly treat macrophage cell models, metformin was generally shown to suppress macrophage inflammatory signals (6). Lymphocyte anti-tumor cytotoxicity is increased upon metformin treatment, both directly (7) and due to reduced PD-L1 expression (8) on cancer cell membrane, or by unlocking cancer immunosuppression by downregulating myeloid-derived suppressor cell functions (9). Finally, metformin has been associated with inhibition of protumorigenic Treg lymphocytes (10). Direct and indirect consequences of metformin treatment are shown in full and dashed lines, respectively. Activation is represented by blue and negative regulation by orange lines.

microenvironment (TME), since recent years have seen a wide collection of reports indicating that metformin, as well as other, more specific, CI inhibitors, may modulate the abundance and the phenotype of non-transformed cell types in a cancer tissue, including endothelial cells, cancer-associated fibroblasts (CAFs), and cells of innate and acquired immunity, such as tumor-associated macrophages (TAMs) and T-lymphocytes (Table 1).

2. Metformin may exert protective effects on the endothelial TME component but inhibits proangiogenic signals in cancer cells

The vascular architecture of a tumor is a consequence of multiple angiogenic signals deriving from either cancer cells, or other cells in the TME, including endothelial cells themselves. Thus, here we distinguish direct and indirect effects of metformin on tumor angiogenesis. Most *in vitro* and *in vivo* studies have reported that metformin affects tumor angiogenesis indirectly, by modulating cancer cell-mediated angiogenic signals (Fig. 1). Metformin has mostly been associated with a decrease in hypoxia-inducible factor 1 alpha (HIF-1α) stability in cancer cells, reducing the expression of HIF1-targeted genes, including *VEGFA*, and thus resulting in slow-growing tumors [2,14,19], often characterized by smaller tumor vessel size [20] and reduced microvessel density [21]. Similarly, we have shown that targeting mitochondrial CI specifically in cancer cells prevents HIF1 activation, and results in immature vasculature [22]. Moreover, reduced HIF1 activity was observed in lung xenografts treated with specific CI inhibitor BAY- 87-2243 [23] and in breast cancer cells treated with the AG311 CI inhibitor under hypoxic conditions [24], suggesting that destabilization of HIF-1α observed in metformin-treated tumors may most likely be attributed to its action on CI. The mechanism behind this phenomenon has been explained, among other, by increased oxygen concentration in cells with lower respiration rate, resulting in HIF-1α destabilization despite extracellular hypoxia [25].

It should be acknowledged that two studies surprisingly reported VEGF upregulation upon metformin treatment, together with increased angiogenesis and tumor growth acceleration [26,27]. One of these studies, performed in melanoma setting, suggests that the proangiogenic and protumorigenic action of metformin may be specific to BRAF-driven transformation, since KRAS-transformed cancer models responded to metformin treatment [27].

The rare studies analyzing the direct effects of metformin on endothelial cells show somewhat contradicting data. Even though not related to cancer, studies in cardiovascular disease models support the concept that metformin might have a direct protective effect on vascular endothelium (Fig. 1) [17,28]. Conversely, by analyzing angiogenesis in a matrigel plug murine model, metformin treatment was associated with a decrease in angiogenesis [29]. The authors also show that metformin downregulates endothelial cell proliferation and invasion *in vitro*, leading to reduced tube formation capacity, and suggest that this is most likely due to activation of AMPK and subsequent ERK downregulation [29]. Indeed, CI inhibition increases AMP/ATP ratio and thus activates AMPK. However, studies in which AMPK activation was induced independently from CI dysfunction, have associated AMPK with upregulation of VEGF in endothelial cells *in vitro* and increased capillary density in ischemic tissue *in vivo,* suggesting a protective effect of AMPK on the endothelium when CI is intact [28]. These seemingly discordant data on the role of metformin in endothelial cell biology might be explained by the fact that CI inhibition, apart from activating AMPK, exerts a series of additional phenomena, such as changes in oxygen concentrations and HIF-1α stability, as well as reduction in lipids availability that are required for endothelial cell proliferation. Moreover, the effects of metformin will highly depend on the dosage. One may hypothesize that the chronic administration of low metformin doses might lead to incomplete inhibition of CI, sufficient to activate AMPK, causing protective effects on the vasculature, as suggested by studies on cardiovascular models. On the other hand, a higher dosage

would lead to a more severe CI dysfunction, changing intracellular oxygen concentrations and inhibiting proangiogenic signalling by preventing HIF1 activation in the endothelial cells.

3. The effect of metformin on CAFs

CAFs have been shown to support cancer progression by supplying angiogenic factors and nutrients, as well as extracellular matrix proteins that serve as scaffold, providing mechanical signals required for cancer cell invasion [30]. Currently available data indicate that the effects triggered by targeting CI seem to be context dependent when regarding CAFs. In an orthotopic pancreatic cancer model, metformin treatment failed to show any difference in activated stromal deposition of collagen I in tumors [31]. Conversely, metformin pre-treated fibroblasts were associated with inhibition of tumor growth *in vivo* and in 3D co-culture models of ovarian cancer, due to NF-kB signalling suppression and subsequent decrease in IL-6 expression in CAFs (Fig. 1) [32]. The authors corroborate their findings by showing a significant decrease in stroma-expressed IL-6 in ovarian cancer patients. On a similar note, metformin treatment of adipocytes suppressed their pro-survival properties, and reduced ovarian cancer migration, proliferation and altered lipid metabolism in co-culture experiments [33]. Moreover, Chen and colleagues report that metformin suppresses CAF-induced pro-clonogenic effect on gastric cancer cell growth *in vitro* [34]. Even though the following study was performed in an unusual experimental setting and does not involve metformin treatment, it is interesting to note that targeting mitochondrial respiration by overexpressing mitochondrial uncoupling proteins reduced cancer cell viability, but increased high-energy nutrient production in the fibroblasts, which in turn supported cancer cell survival in a paracrine fashion [35]. In line with these data, we recently observed that genetic targeting of CI is associated with a more abundant stromal component [22], suggesting that CI inhibition in tumors may result in metabolic symbiosis through which CAFs support CI-deficient cancer cells to overcome metabolic constraints.

4. Contrasting effects of metformin on TAMs

Depending on their function, in the context of cancer, macrophages may be roughly divided into two subpopulations, namely the anti-tumorigenic proinflammatory M1, and the M2 that are activated in the wound-healing process and have been shown to support tumor progression. Studies analyzing the direct effect of metformin on macrophages have in general associated metformin treatment with the inhibition of macrophage-mediated inflammatory signals (Fig. 1). For example, several *in vitro* studies showed that metformin downregulates lipopolysaccharide (LPS)-induced NF-kB signalling in murine RAW macrophages [36,37] and decreases LPS-induced cytokine secretion [38]. Of note, studies mainly working with *ex vivo* LPS- or palmitate-stimulated bone marrow-derived macrophages (BMDMs) showed that, while reducing inflammatory cytokine expression (such as TNF-α, IL-6, IL-1b), metformin increased expression of wound-healing IL-10 cytokine and correlated with increase of M2 markers [37,39–42], suggesting metformin might induce pro-tumorigenic macrophage phenotype. Nevertheless, in the cancer setting, metformin treatment has most often been associated with inhibition of the macrophage pro-tumorigenic role, indicating a possible indirect effect of metformin on TAM function due to changes occurring in metformin-treated cancer cells (Fig. 1). In line with this hypothesis, Chen and colleagues have observed that metformin-mediated increase in M2 population markers was lacking when metformin-treated macrophages were co-cultured with cancer cells [43]. Studies working on syngenic immunocompetent mouse cancer models showed metformin is associated with decreased number of TAMs in the tumor tissue [44], as well as with skewing TAM polarization towards anti-tumorigenic M1 population [45,46]. In particular, Liu reported that metformin inhibits prostate cancer cell-mediated macrophage migration into the tumor [44]. Others have associated metformin with decrease in M2 macrophage population, since Arg1 downregulation was observed upon metformin treatment in breast cancer macrophages [46] and reduced number of CD206-positive TAMs was found in metformin-treated Lewis lung carcinoma model [45]. Interestingly, the two latter studies reported either no difference [45] or even an increase in the absolute TAM number in metformin-treated tumor mass [46], the latter being in accordance with our latest data, showing an abundance of TAMs in CI-deficient and metformin-treated xeongrafts [22].

There is little insight on what could be the mechanism through which metformin modulates macrophage function. Most studies correlate metformin treatment to AMPK activation, which may then reduce NF-kB, JNK and STAT signalling pathways involved in inflammatory response [42,47]. However, AMPK-independent downregulation of NF-kB by metformin has been described in some settings [36,37], indicating that signals other than AMPK sensing regulate metformin-mediated inflammatory response. It is important to note that such downregulation of inflammatory signalling may occur either in the macrophage or in a cancer cell, respectively triggering direct or indirect effects of metformin on the final TAM phenotype (Fig. 1). Moreover, hypoxia and spatial distance from the perfusing vessel have been shown to define TAM polarization, in a way that hypoxic regions are associated with high expression of the Arg1 M2 macrophage marker [48]. In this context, it is important to acknowledge that metformin may reduce respiration in cancer cells, preventing formation of hypoxic regions in the tumor [14], possibly explaining why metformin treatment reduces M2 markers expression (Fig. 1). [45–47]. Nonetheless, regardless of the reports indicating association of metformin with antitumorigenic M1 macrophage phenotype, targeting TAMs in colorectal cancer xenografts has shown to potentiate metformin efficacy, whereby we hypothesize that CI inhibition in cancer cells leads to recruitment of TAMs to support tumor growth [22].

5. Metformin inhibits immunosuppressive responses by boosting cytotoxic T-lymphocyte functions

Cancer cells are able to suppress the cytotoxic effects of lymphocytes by various mechanisms, the most well-known being the overexpression of programmed death ligand 1 (PD-L1), which causes cytotoxic T-cell exhaustion, resulting in immunosuppression and cancer cell survival [49]. On the other hand, tumors often harbor high numbers of protumorigenic regulatory T-cells (Treg), which support tumor growth by promoting wound-healing-like signals [50]. The current literature generally agrees that metformin boosts anti-tumor adaptive immune response (Fig. 1). Increased tumor infiltrating lymphocyte (TIL) abundance and enhanced cytotoxic T-cell functions were described both in primary tumor and in metastatic experimental settings upon metformin treatment [51–53]. Eikawa was first to show that, in contrast to the anti-survival effect ascribed to metformin regarding cancer cell viability, metformin treatment may protect TILs from apoptosis [51]. Moreover, metformin was shown to increase TIL multifunctionality (triple inflammatory cytokine production: IL-2, TNF-α, IFN-γ), regardless of their PD-L1 status, a phenomenon which could be abrogated by the AMPK inhibitor compound C (Fig. 1). Since metformin should decrease mitochondrial respiration simultaneously in T-lymphocytes and cancer cells, leaving glycolysis as the common metabolic engine in both cell types, and thus promoting competition for glucose, it is intuitive to hypothesize such avidity for sugar would lead to glucose shortage in the TME and eventually block cytotoxic T-cell effector function. Nevertheless, the study which predominantly focused on RLmale1 tumors in Balb/c mice, showed metformin to exhibit anti-tumorigenic effects via direct action on CD8+ TILs, as it reduced their exhaustion, raising the question about how metformin promotes cytotoxic T-cell phenotype [51]. One possible explanation comes from a study of cancer progression in obese models which suggested that metformin in combination

with targeting PlGF/VEGF1R pathway allows a higher influx of cytotoxic T cells into the tumor site due to increased perfusion [54]. This hypothesis was drawn also for the cytotoxic T and NK cells in the context of pancreatic cancer where high T-cell numbers in metformin-treated masses have been associated with improved vascularization and reduced dysplasia [55]. Interestingly, hypoxia was shown to reduce IFN-γ expression and T-cell cytolytic activity against cancer cells, whereas elevating intracellular oxygen concentration by metformin resulted in increased T-cell activation, suggesting that hypoxic signalling modulates T-cell phenotype regardless of the tumor perfusion status [55]. Moreover, metformin was found to downregulate HIF1 in ovarian cancer myeloid-derived suppressor cells (MDSCs), decreasing their immunosuppressive activity and improving cytotoxic T-cell functions, pointing out to an indirect effect of hypoxia on the anti-tumor T-cell activity (Fig. 1) [56]. Of note, a reduced MDSCs immunosuppressive action on T-cells was observed also upon treatment with biguanidine phenformin [57].

Another mechanism through which metformin promotes cytotoxic T-cell phenotype was recently uncovered by Cha and colleagues who, in a detailed and convincing set of experiments, explain that high CD8+ T-cell mediated cytotoxic activity in metformin-treated 4T1 breast tumors in BALB/c is due to downregulation of PD-L1 in cancer cells. In particular, AMPK activated by metformin caused endoplasmic-reticulum associated degradation of PD-L1, prevented its processing to Golgi and decreased PD-L1 localization on the cancer cell membrane, eventually boosting the effect of cytotoxic T lymphocytes [52]. Of note, both Eikawa and Cha report that metformin did not have an anti-tumorigenic effect in immunodeficient SCID mice [51,52], which prompted the authors to attribute the anti-tumorigenic properties of the drug mainly to T-cell activity. However, their findings should not be generalized, since many studies in nude immunodeficient mice concur on the anti-tumorigenic effect of metformin [2,58]. Different outcomes could be due to the fact that Eikawa and Cha monitored early response to the drug (10–20 days). Moreover, diverse metformin effects are most likely dependent on the oncogene driving the transformation, as it was observed, for example, that its antitumorigenic potential is modulated based on whether cancer cell transformation is associated or not with an inflammatory signature [59].

Apart from promoting cytotoxic T-cell functions, it has been reported that the immune cell-mediated anti-tumorigenic effects of metformin may be exerted also by downregulating pro-tumorigenic lymphocytes. In particular, Zhao et al, in a study on orthotopic hepatocellular carcinoma, showed that metformin prevents differentiation of a specific subtype of T helper cells (Th1 and Th17) producing wound-healing-associated cytokine IL-22, which eventually leads to reduction of hepatocellular cancer cell growth in BALB/c livers [60]. Moreover, metformin was reported to prevent Treg infiltration into tumors, via mammalian Target of rapamycin complex (mTORC1) inhibition and subsequent Foxp3 downregulation, normally required for Treg differentiation [61].

6. Conclusions

The evidence collected so far clearly shows that, apart from blocking cancer cell proliferation, metformin may influence tumor progression by modulating TME. This may be achieved indirectly, as metformin-induced metabolic changes in cancer cells reflect on the phenotype of non-malignant cells in a tumor mass. For example, by elevating oxygen concentration in cancer cells, metformin causes downregulation of HIF1-mediated endothelial cell proliferation, and by reducing cancer cell energy charge it promotes PD-L1 degradation in cancer cells, boosting cytotoxic T-cells (Fig. 1). On the other hand, metformin has also shown to directly skew the phenotype of TME populations, such as macrophages and T-cells, by modulating their cytokine production, for example, via the NF-kB pathway.

We are only starting to understand the complexity of the effects metformin may have on different TME populations, which were reported to depend not only on the cell and tissue type, but also on parameters such as the hypoxic status of a tumor mass or the oncogene driving the progression. Moreover, since metformin has recently shown the optimal antitumorigenic performance in hypoglycaemia [18], and nutrient availability is known to significantly skew the functions of non-malignant cells in a tumor [62], it will be particularly important to evaluate the effect of metformin on TME populations when the treatment is implemented upon fasting conditions.

As the molecular and biochemical mechanisms underlying the metformin mode of action are emerging, it is clear they will need to be investigated not only in cancer cells, but also in the non-malignant populations of TME. For the time being, it is reasonable to conclude that metformin may trigger both antitumorigenic and protumorigenic effects in the case of endothelial cells and macrophages, suggesting that combinatorial therapeutic approaches should be foreseen in certain cases to increase its efficacy. On the other hand, the current literature generally agrees on the fact that metformin promotes cytotoxic functions of T lymphocytes, underlining the importance of using immunocompetent models and patient-derived data to draw conclusions on the final outcome of metformin treatment in cancer.

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Declaration of interest

The authors have nothing to disclose.

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