Review

Tumor Immune Evasion Induced by Dysregulation of Erythroid Progenitor Cells Development

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Simple Summary: Tumor immune evasion is one of the hallmarks of tumor progression that enables tumor growth despite the activity of the host immune system. It is mediated by various types of cells. Recently, immature red blood cells called erythroid progenitor cells (EPCs) were identified as regulators of the immune response in cancer. EPCs expand in cancer as a result of dysregulated erythropoiesis and potently suppress the immune response. Thus, targeting dysregulated EPC differentiation appears to be a promising therapeutic strategy.

Abstract: Cancer cells harness normal cells to facilitate tumor growth and metastasis. Within this complex network of interactions, the establishment and maintenance of immune evasion mechanisms are crucial for cancer progression. The escape from the immune surveillance results from multiple independent mechanisms. Recent studies revealed that besides well-described myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) or regulatory T-cells (Tregs), erythroid progenitor cells (EPCs) play an important role in the regulation of immune response and tumor progression. EPCs are immature erythroid cells that differentiate into oxygen-transporting red blood cells. They expand in the extramedullary sites, including the spleen, as well as infiltrate tumors. EPCs in cancer produce reactive oxygen species (ROS), transforming growth factor β (TGF-β), interleukin-10 (IL-10) and express programmed death-ligand 1 (PD-L1) and potently suppress T-cells. Thus, EPCs regulate antitumor, antiviral, and antimicrobial immunity, leading to immune suppression. Moreover, EPCs promote tumor growth by the secretion of growth factors, including artemin. The expansion of EPCs in cancer is an effect of the dysregulation of erythropoiesis, leading to the differentiation arrest and enrichment of early-stage EPCs. Therefore, anemia treatment, targeting ineffective erythropoiesis, and the promotion of EPC differentiation are promising strategies to reduce cancer-induced immunosuppression and the tumor-promoting effects of EPCs.

Keywords: immune evasion; erythroid progenitor cells; CD71+ erythroid cells; erythropoiesis; anemia; Ter-cells; ineffective erythropoiesis

1. Introduction

Cancer immunotherapy has strongly changed the therapeutic landscape in clinical oncology, leading to significant improvements in cancer patients survival [1]. However, despite the induction of durable responses in an unprecedented percentage of cancer patients, the majority still do not respond to the treatment and eventually progress to refractory disease. There are several defined causes of immunotherapy resistance, including low tumor mutational burden [2], impaired antigen presentation by the major histocompatibility complex (MHC) proteins [3], loss of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) pathway genes [4,5], as well as the development of immunosuppressive tumor microenvironment (TME) [6,7].
TME is composed of many types of cells that regulate tumor growth and progression [8]. The role of regulatory T-cells (Tregs) [9], myeloid-derived suppressor cells (MDSCs) [10], tumor-associated macrophages (TAMs) [11], tumor-associated neutrophils (TANs) [12], and cancer-associated fibroblasts (CAFs) [13] in the regulation of anti-tumor immune response has been established by many years of research (Table 1). Recent reports point to another population of cells, i.e., erythroid progenitor cells (EPCs), that regulate local and systemic immunity in cancer. These cells use similar mechanisms to immune cells and are crucial in the regulation of immune response and cancer progression.

**Table 1.** Immunomodulatory cells in cancer and their mechanisms of immune regulation.

| Cells                              | Mechanisms          | Effects                                             | Ref |
|------------------------------------|---------------------|-----------------------------------------------------|-----|
| Regulatory T-cells (Tregs)         | IL-10               | T-cell suppression                                  | [14]|
|                                    | IL-2 consumption    | T-cell suppression                                  | [15]|
|                                    | COX-2 and PGE2      | T-cell suppression                                  | [16]|
|                                    | Adenosine           | T-cell suppression                                  | [17]|
|                                    | ARG1                | T-cell suppression                                  | [18]|
|                                    | IDO                 | T-cell suppression, Tregs induction, NK cell suppression | [19,20]|
| Myeloid-derived suppressor cells (MDSCs) | PD-L1/PD-1         | T-cell suppression                                  | [21]|
|                                    | IL-10               | T-cell suppression                                  | [22]|
|                                    | TGF-β               | T-cell suppression                                  | [22]|
|                                    | CD40/CD40L          | T-cell suppression                                  | [22]|
|                                    | Depletion of cystine and cysteine ROS | T-cell suppression | [24]|
|                                    | Free radical peroxynitrite | Resistance to cytotoxic T-cells                  | [26]|
| Tumor associated macrophages (TAMs) | PD-L1/PD-1         | Decreased phagocytosis                              | [27]|
|                                    | ARG1                | T-cell suppression                                  | [28]|
|                                    | IL-10               | T-cell suppression                                  | [29]|
|                                    | IL-1β               | Induction of the protumor phenotype                 | [30,31]|
|                                    | IL-12               | Induction of T-cell response                        | [32]|
|                                    | TNF-α               | Induction of anti-tumor response                    | [33]|
| Tumor associated neutrophils (TANs) | ARG1                | T-cell suppression                                  | [18,28]|
|                                    | NOS                 | T-cell suppression                                  | [34,35]|
|                                    | PD-L1/PD-1          | T-cell suppression                                  | [36]|
| Cancer associated fibroblasts (CAFs) | PD-L1/PD-1         | T-cell suppression                                  | [37]|
|                                    | FasL, PD-L2         | T-cell suppression                                  | [38]|
|                                    | IL-6                | Induction of PD-L1+ TANs                            | [39]|
|                                    | Chemokines          | MDSC infiltration                                   | [40]|
| Erythroid progenitor cells (EPCs)   | ROS                 | T-cell suppression                                  | [41,42]|
|                                    | IL-10               | T-cell suppression                                  | [42]|
|                                    | PD-L1/PD-1          | T-cell suppression                                  | [43]|
|                                    | TGF-β               | T-cell suppression                                  | [42]|

ARG1—arginase 1, COX-2—cyclooxygenase-2, FasL—Fas ligand (CD95L, CD178), IDO—Indoleamine-pyrrole 2,3-dioxygenase, IL—interleukin, NK—natural killer, NOS—nitric oxide synthase, PD-1—programmed cell death 1, PD-L1—programmed death-ligand 1, PGE2—Prostaglandin E2, ROS—reactive oxygen species, TGF-β—transforming growth factor β, TNF-α—tumor necrosis factor α.
In this review, we discuss the role of the dysregulation of erythropoiesis by cancer cells to induce immune evasion and promote cancer progression.

2. Regulation of Erythropoiesis

The differentiation of hematopoietic stem cells (HSCs) to erythroid cells is a stepwise process strictly regulated by multiple intrinsic and extrinsic factors (Table 2), which results in the production of over $2 \times 10^{11}$ red blood cells (RBCs) per day and allows for the maintenance of erythroid homeostasis [44–48]. This complex net of interactions provides adequate production of RBCs depending on the body’s needs. Insufficient oxygen supply to the peripheral tissues resulting in hypoxia is a key trigger of increased erythropoiesis, which is regulated by the increased production of erythropoietin (EPO) in the kidney peritubular fibroblasts and liver interstitial cells and hepatocytes [49].

Table 2. Regulation of erythropoiesis.

| Factor   | Role in Erythropoiesis                        | Dysregulation in Cancer                                                                 | References |
|----------|-----------------------------------------------|----------------------------------------------------------------------------------------|------------|
| SCF      | Growth factors regulating early stages of erythropoiesis | Production in TME                                                                     | [50,51]    |
| G-CSF    |                                               | Increased serum concentration                                                          | [52]       |
| IL-3     |                                               |                                                                                      | [53]       |
| EPO      | Growth factors regulating late stages of erythropoiesis | Increased serum concentration                                                          | [54]       |
| GDF11    |                                               | Production in TME                                                                      | [55]       |
| Activin A|                                               | Production in TME                                                                      | [56]       |
| GATA1    | Crucial TFs regulating erythropoiesis         | Decreased expression in EPCs in cancer                                                | [57–59]    |
| STAT5    |                                               | Increased in EPCs in MPNs                                                             |            |
| MCL-1    | Survival factors for erythroid cells         | Decreased in EPCs in iron deficiency                                                  | [60,61]    |
| BCL-xL   |                                               |                                                                                      |            |
| HSP70    |                                               |                                                                                      |            |
| TGF-β    | Negative regulators of erythropoiesis        | Production in TME                                                                      | [62]       |
| SMAD signaling |                                               | Increased concentration                                                               | [62]       |
| FasL     |                                               | Increased level in EPCs in cancer                                                    | [59,63]    |
| Fas      |                                               | High expression on cancer cells                                                      |            |
| Vitamin B12 |                                               | Decreased in a subset of patients                                                    | [64]       |
| Folic Acid|                                               | Decreased in a subset of patients                                                    | [64]       |
| Copper   | Essential vitamins, trace elements, and iron-metabolism proteins | Increased concentration                                                               | [65]       |
| Iron     |                                               | Decreased in a subset of patients                                                    | [66]       |
| Ferritin |                                               | Decreased or increased                                                                | [66]       |
| Transferrin |                                               | Decreased in a subset of patients                                                    | [66]       |
| Ferroportin |                                               | Increased expression                                                                  | [67]       |
| Hepcidin |                                               | Increased concentration                                                               | [68]       |

MPN—myeloproliferative neoplasm, TF—transcription factor, TME—tumor microenvironment.

HSCs reside in a unique niche that is created and regulated by various cell types, growth factors, and chemokines [69]. The commitment of HSCs to erythroid lineage begins with the differentiation to a multipotent megakaryocyte–erythroid progenitor cell (MEP), followed by a burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid
(CFU-E). During terminal erythropoiesis, CFU-E differentiates into proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts, and orthochromatic erythroblasts that expel their nuclei and generate reticulocytes [70]. Reticulocytes are released to the circulation, where they mature to RBCs within a few days. In healthy humans, erythroblasts represent about 20–30% of nucleated cells in the bone marrow [71,72].

The first steps of erythropoiesis are regulated by hematopoietic cytokines including stem cell factor (SCF), interleukin 3 (IL-3), insulin-like growth factor 1 (IGF-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [73–75]. Further erythroid cell differentiation is regulated mainly by EPO [45,76,77]. The impairment of steady-state erythropoiesis triggers stress erythropoiesis that maintains erythroid homeostasis. Stress erythropoiesis is regulated by additional factors including hypoxia, bone morphogenetic protein 4 (BMP4), Hedgehog, glucocorticoids, and peroxisome proliferator-activated receptor α (PPAR-α) [78,79].

Cell lineage specification is regulated through defined transcriptional programs. It is well established that a zinc-finger transcription factor GATA1 is a master transcriptional regulator of differentiation toward erythroid lineage [80]. It is induced at the very early stages of erythropoiesis and is responsible for the regulation of all known erythroid genes [80]. Thus, GATA1 is necessary for erythropoiesis and its lack cannot be compensated as Gata1 knockout mice fail to generate mature RBCs [81]. Therefore, the cleavage of GATA1 is a key mechanism of erythropoiesis regulation. GATA1 is cleaved by caspases, primarily caspase-3, which is activated in the nucleus of terminally differentiating erythroid cells to enable maturation to RBCs [80,82,83]. Nonetheless, the activation of caspases and GATA1 degradation at earlier stages of differentiation induces differentiation arrest and apoptosis. Therefore, GATA1 is protected from degradation in early-stage EPCs by EPO signaling, p19INK4d cyclin-dependent kinase inhibitor, and HSP70 protein chaperone [76,82,84].

3. Erythroid Progenitor Cells as Immune Regulators

EPCs are predominantly erythroblasts and reticulocytes that differentiate into mature RBCs. EPCs are characterized by the expression of transferrin receptor 1 (CD71) and glycophorin A (CD235a) in humans, and CD71 and TER119 in mice [85]. For many years, EPCs were considered to be solely erythrocytes precursors, without any other significant functions in the human body. However, recent studies revealed the importance of the previously neglected role of EPCs.

Immunomodulatory functions of EPCs were described for the first time in neonates, which are characterized by a physiological enrichment of EPCs in extramedullary sites, including the spleen, liver, and peripheral blood [86]. Neonatal EPCs express arginase-2 (ARG2), \( \text{L-arginine degrading enzyme} \), and secrete transforming growth factor β (TGF-β), leading to the suppression of cytokine production by myeloid cells [86] and the promotion of T-cell differentiation toward Tregs cells [87]. Despite initial hypotheses that only neonatal EPCs have significant immunoregulatory properties [86], further research expanded our knowledge and revealed that these properties are a general feature of EPCs. The regulation of immune cells by erythroid cells was described for EPCs induced by pregnancy [88], systemic inflammation [89], HIV infection [90], COVID-19 [91], and anemia [92].

EPCs in different conditions modulate immune response via various mechanisms (Table 3). Recent studies also demonstrated that EPCs that expand during cancer progression possess significant immunomodulatory properties and promote tumor growth.
Table 3. Mechanisms of immunomodulatory functions of EPCs.

| Source          | Mechanism | Effect                                      | Mouse | Humans | Ref.   |
|-----------------|-----------|---------------------------------------------|-------|--------|--------|
| Neonates        | ARG2      | ↓ cytokine production by myeloid cells       | +     | +      | [86,93]|
|                 | TGF-β     | ↑ Tregs differentiation                      | +     | +      | [87]   |
|                 | ROS       | ↓ cytokine production by myeloid cells       | -     | +      | [94]   |
|                 | PD-1/PD-L1| ↓ cytokine production by T-cells             | +     | +      | [88]   |
| Pregnancy       | ARG2      | ↓ cytokine production by myeloid cells       | +     | +      | [24,93]|
|                 | TGF-β     | ↑ Tregs differentiation                      | n.d.  | +      | [93]   |
|                 | ROS       | ↓ cytokine production by myeloid cells       | n.d.  | +      | [93]   |
|                 | PD-1/PD-L1| ↓ cytokine production by T-cells             | +     | +      | [88]   |
| Inflammatory    | EPCs      | ↓ cytokine production by red pulp macrophages| +     | n.d.   | [89]   |
| diseases        | phagocytosis |                                         |       |        |        |
| HIV-infected    | ROS       | ↑ HIV replication in T-cells                | n.d.  | +      | [90]   |
| patients        |           | ↑ HIV trans-infection                       |       |        |        |
| COVID-19 patients| ARG1     | ↓ cytokine production by T-cells             | n.d.  | +      | [95]   |
|                 | ARG2      | ↓ cytokine production by T-cells             | n.d.  | +      | [95]   |
|                 | ROS       | ↓ cytokine production by T-cells             | n.d.  | +      | [95]   |
| Anemia          | ARG1      | ↓ cytokine production by T-cells             | -     | +      | [92]   |
|                 | ARG2      | ↓ cytokine production by T-cells             | +     | +      | [92]   |
|                 | ROS       | ↓ cytokine production by T-cells             | +     | +      | [92]   |
| Cancer          | TGF-β     | ↓ T-cells proliferation, ↓ cytokine production by T-cells | n.d.  | +      | [42]   |
|                 | ROS       | ↓ T-cell proliferation, ↓ cytokine production by T-cells | +     | +      | [41,42]|
|                 | PD-L1/PD-1| ↓ cytokine production by T-cells             | +     | +      | [43]   |
|                 | IL-10     | ↓ T-cell proliferation, ↓ cytokine production by T-cells | n.d.  | +      | [42]   |

↑—promoted, ↓—suppressed, n.d.—no data, - — no role, +—reported mechanism.

4. The Role of Erythroid Progenitor Cells in Cancer

Cancer progression is associated with the suppression of immune response that enables tumor growth and leads to increased susceptibility to infections in patients with advanced disease [96]. It is caused by the remodeling of the immune cell landscape that impairs not only a local anti-tumor response, but also systemic antibacterial and antiviral immunity [97]. Cancer cells and tumor-associated stromal cells reprogram hematopoiesis and promote the polarization of immune cells toward suppressive phenotypes. In cancer, the spleen is a key organ of extramedullary hematopoiesis and is responsible for the production of suppressive immune cells [98]. It is well established that cancer dysregulates hematopoiesis to generate MDSCs that suppress antitumor response [10,99]. However, during tumor progression, immune cells in the murine spleen are vastly outnumbered by another type of cells, EPCs [41,62]. Moreover, substantial EPC expansion is observed in the peripheral blood and the liver of tumor-bearing mice and cancer patients. EPCs also infiltrate murine and human tumors, and their frequency in TME is much higher than that of MDSCs or Treg cells [41–43,62].

Similar to neonatal counterparts, EPCs induced by cancer were found to potently suppress immune response (Figure 1). The proliferation and cytotoxicity of CD8$^+$ T-cells, as well as the proliferation of CD4$^+$ T-cells and T_{H1} differentiation, are inhibited by tumor-
induced murine EPCs [41]. In murine models, the depletion of EPCs with anti-CD71 antibody inhibits tumor growth [43]. Likewise, EPCs isolated from peripheral blood of cancer patients or human tumors potently suppress T-cell proliferation and the production of IFN-γ via paracrine and direct cell-to-cell contact manner [41,42].

**Figure 1.** The role of erythroid progenitor cells (EPCs) in cancer. During disease progression, EPCs expand in the extramedullary sites, including the spleen. Moreover, EPCs are abundant in the peripheral blood of cancer patients and infiltrate the tumor microenvironment. Early-stage CD45⁺ EPCs use (1) reactive oxygen species (ROS), (2) interleukin-10 (IL-10), (3) transforming growth factor β (TGF-β), and (4) programmed death-ligand 1 (PD-L1) to modulate the immune response. EPCs inhibit (A) T-cell activation, (B) production of interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), (C) T-cell proliferation, and (D) cytotoxicity of CD8⁺ T-cells. More mature CD45⁻ EPCs regulate cancer progression by (5) secretion of a neurotropic factor, artemin. These late-stage EPCs, called Ter-cells, promote (E) tumor cell migration and invasiveness as well as (F) tumor growth and cell proliferation.

Erythropoiesis is a continuous process by which erythroid cells change their characteristics to differentiate into specialized oxygen-transporting RBCs. The transcriptional profile [41,42,100–107] and cell proteome [106,108–111] substantially change during erythroid maturation. Growing evidence indicates that the role of EPCs in cancer changes with maturation (Table 4). During differentiation, EPCs lose expression of CD45, a pan-leukocyte marker [112]. Therefore, CD45 may be used as a marker of early-stage EPCs [41]. In tumor-bearing mice, CD45⁺ EPCs constitute over 40% of EPCs and are predominantly responsible for the immunosuppressive effects of EPCs [41]. These early-stage EPCs were found to potently suppress T-cells, in contrast to more mature CD45⁻ erythroid cells [41,62]. In mice, the suppressive capacity of CD45⁺ EPCs falls between Tregs and MDSCs [41], but in humans, CD45⁺ EPCs are even more potent immunosuppressors than both Tregs and MDSCs [42].
Table 4. Differences in immune-related mediators between early-stage and late-stage EPCs [41–43,62].

| Feature                  | Early-Stage EPCs (CD45⁺) | Late-Stage EPCs (CD45⁻) |
|--------------------------|--------------------------|-------------------------|
| ROS level                | ↑                        | ↓                       |
| IL-10                    | ↑                        | ↓                       |
| TGF-β                    | ↑                        | ↓                       |
| ROS pathway              | ↑                        | ↓                       |
| IL-10 pathway            | ↑                        | ↓                       |
| TGF-β pathway            | ↑                        | ↓                       |
| PD-1/PD-L1               | n.d.                     | n.d.                    |
| ARG2                     | n.d.                     | n.d.                    |

↑—increased, ↓—decreased, n.d.—no data.

Transcriptional analysis revealed a close resemblance between CD45⁺ EPCs and MDSCs and enrichment in the reactive oxygen species (ROS) pathway in CD45⁺ EPCs [41]. Early stage CD45⁺ EPCs have upregulated expression of NADPH oxidase (NOX) family members [41,42], crucial ROS-generating NADPH oxidases [113]. As a result, they have increased ROS levels compared to CD45⁻ counterparts [41,42]. Although ROS are required for T-cell activation, excessive ROS levels impair T-cell immunity [114]. Thus, ROS are a well-established mechanism of T-cell suppression by MDSCs [115]. Similarly, EPCs were found to suppress T-cells in a ROS-dependent manner. Apocynin, an NADPH oxidase inhibitor, as well as N-acetylcysteine, an ROS scavenger, diminished the suppressive effects of EPCs [41,42,116]. The infiltration of EPCs to TME probably contributes to the high ROS levels triggering oxidative stress. High ROS levels in TME impair the functions of tumor-infiltrating lymphocytes and dendritic cells, while promoting the recruitment and accumulation of Tregs and MDSCs [117]. However, ROS inhibition did not restore T-cell functions completely [41]. Further studies revealed that CD45⁺ EPCs induced by cancer use multiple additional immunoregulatory mechanisms, including IL-10, TGF-β, and PD-1/PD-L1 [42,43]. Thus, the immunoregulatory functions of EPCs rely on many mechanisms identified for immunosuppressive cells in TME (Table 1).

It seems that EPCs impair both anti-tumor immunity and systemic immune response to pathogens. In mice, CD45⁺ EPCs potently inhibit the antigen-specific response of tumor-infiltrating cytotoxic T-cells [41]. The transfer of CD45⁺ EPCs into tumor-bearing mice accelerated tumor growth [41], confirming the suppression of anti-tumor response by EPCs. Likewise, CD45⁺ EPCs suppressed proliferation and cytokine production by tumor-infiltrating T-cells from cancer patients [42].

Importantly, the expansion of EPCs in cancer is most remarkable in the spleen (Table 3), which is the largest secondary lymphoid organ involved in the development of systemic immune response to blood-borne antigens [118]. Similarly to neonates that are also characterized by the expansion of EPCs in the spleen [86], adult tumor-bearing mice have increased susceptibility to viral and bacterial infections compared to healthy mice [41].

Ex vivo, EPCs suppressed antigen-specific cytotoxic T-cells [41]. In vivo, the depletion of EPCs with anti-CD71 antibody restored the suppressed proliferation of virus-specific CD8⁺ T-cells, restoring anti-viral immunity in tumor-bearing mice while the transfer of CD45⁺ EPCs potentiated the suppression of immune response [41]. In humans, anemic cancer patients have higher EPC numbers and increased Epstein–Barr viral (EBV) load due to suppressed anti-viral immunity [41]. These latter findings suggest that as in mice, EPCs suppress a systemic immune response in cancer patients.

It was suggested that the suppressive properties of EPCs may be restricted to stress erythropoiesis-induced EPCs. However, CD45⁺ EPCs isolated from the spleen, liver as well as bone marrow of the tumor-bearing mice suppressed T-cells to a similar extent [41]. Moreover, steady-state EPCs from human bone marrow also suppress T-cells [92]. Nonetheless, there are significant differences in the expression of immunomodulatory molecules, including PD-L1, between EPCs isolated from the bone marrow, spleen, and TME [43]. This suggests that the differences in EPC properties may result from stimulation with some
factors, presumably cytokines or TME components, which may enhance or diminish the immunosuppressive properties of EPCs.

**Tumor-Promoting Role of CD45− EPCs**

The majority of tumor-induced EPCs are CD45− [41,62]. While early-stage CD45+EPCs potently suppress the immune response, more mature CD45− EPCs lack this capacity (Table 4). However, the transfer of CD45− EPCs also promotes tumor growth and decreases the survival of tumor-bearing mice [62].

These tumor-induced splenic CD45− EPCs were called Ter-Cells [62]. They are a population of late-stage EPCs as they have a high nucleus/cytoplasm ratio, scant cytoplasm, dense chromatin, and few organelles, as well as lacking the expression of the major histocompatibility complex (MHC) class I [62], a marker of mature erythroid cells [119]. In contrast to early-stage EPCs, CD45− EPCs do not influence T-cell proliferation, dendritic cell activation, and cytokine secretion as well as fail to induce Tregs [62]. Moreover, CD45− EPCs have very low or undetectable levels of immune-related mediators, including IL-10, TGF-β, IL-4, prostaglandin E2 (PGE-2), and ROS [41,42,62]. Therefore, CD45− EPCs do not promote tumor growth by inhibiting the anti-tumor response.

Transcriptional analysis revealed marked overexpression of a neurotrophic factor artemin in CD45− EPCs [62]. The physiological role of artemin involves the regulation of neuronal survival, maintenance, and differentiation [120]. Artemin also has protumorigenic activity and promotes cancer cell survival, proliferation, migration, and invasiveness [62,121–123]. In murine models, it promotes tumor growth and accelerates disease progression [62]. Artemin activates the glial cell-derived neurotrophic factor (GDNF) family receptor alpha-3 (GFRα3) and its co-receptor RET on cancer cells. Downstream signaling of artemin promotes the phosphorylation of extracellular signal-regulated kinase (ERK), protein kinase B (AKT), and caspase-9, promoting proliferation and invasiveness, while preventing apoptosis in tumor cells, even induced by the therapy [62]. The same effects are exerted by artemin-secreting CD45− EPCs. Thus, the reduction in EPC expansion reduces the increase in the artemin concentration in the serum and decreases tumor growth [62]. Artemin-expressing CD45− EPCs were also detected in the spleens of patients with hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma (PDAC) [62,123], which suggests their role in cancer patients.

Moreover, these differences are also manifested by their localization. While immunomodulatory early-stage EPCs accumulate in the spleen and intensively infiltrate TME, tumor-promoting late-stage EPCs are detected mainly in the spleen where they secrete artemin into circulation [41,42,62,123]. Collectively, early-stage and late-stage EPCs differ substantially regarding their gene expression profile, level of immunomodulatory mediators, and their role in promoting cancer progression (Table 5).

| Table 5. Different role of early-stage and late-stage EPCs in cancer [41–43,62,116,123]. |
|---|---|---|
| Process | Early-Stage EPCs (CD45+) | Late-Stage EPCs (CD45−) |
| T-cell proliferation | ↓ suppressed | ↔ no effect |
| Production of IFN-γ by T-cells | ↓ suppressed | ↔ no effect |
| Production of TNF-α by T-cells | ↓ suppressed | ↔ no effect |
| CD8+ T-cells cytotoxicity | ↓ suppressed | ↔ no effect |
| Dendritic cells activation | n.d. | ↔ no effect |
| Production of IL-6 and IL-12 by dendritic cells | n.d. | ↔ no effect |
| Tregs induction | n.d. | ↔ no effect |
| Anti-tumor immune response | ↓ suppressed | ↔ no effect |
| Activation of signaling pathways in tumor cells | n.d. | ↑ promoted |
| Regulation of cancer cell metabolism | ↑ promoted | n.d. |
| Tumor cells proliferation | n.d. | ↑ promoted |
| Tumor cells invasiveness | n.d. | ↑ promoted |
| Tumor growth | ↑ promoted | ↑ promoted |

↑—promoted, ↓—suppressed, ↔—no effect, n.d.—no data.
5. Expansion of Erythroid Progenitor Cells

EPCs predominantly occupy niches in the bone marrow where they differentiate into RBCs. However, EPC frequency in the steady-state bone marrow is relatively low, especially when compared to mature erythrocytes. In healthy individuals, EPCs are not detected in extramedullary sites, besides a small percentage of reticulocytes in peripheral blood [124]. However, under several conditions, EPCs substantially expand in the bone marrow as well as in extramedullary sites.

The expansion of EPCs is physiological in neonates and during pregnancy [88,93,125,126]. In neonates, EPCs accumulate in the extramedullary sites due to insufficient bone marrow erythropoiesis during the first days of life [125,126]. During pregnancy, extramedullary erythropoiesis enables the production of sufficient numbers of erythrocytes [88]. Moreover, the expansion of EPCs is also observed in anemic patients as a mechanism increasing oxygen transport [92,127]. Recent studies revealed that extramedullary erythropoiesis and EPC expansion may also be a part of the inflammatory response [128–130]. A recent analysis of blood transcriptome revealed that the signature of immature erythroid cells is also associated with severe respiratory syncytial virus (RSV) infection, pharmacological immunosuppression, and late-stage cancer [131].

In tumor-bearing mice, EPCs expand during tumor progression in many extramedullary organs (Table 6), predominantly the spleen, liver, and peripheral blood, as well as infiltrate tumors [41,43,62]. In humans, EPCs were detected in the spleen, TME, and peripheral blood of cancer patients [41,62,123,131]. In general, anemia severity correlates with the frequency of EPCs [41]. In some cases, the expansion of EPCs in peripheral blood is so substantial that it causes a so-called leukoerythroblastic reaction [132,133].

| Table 6. The frequency of EPCs in tumor-bearing mice and cancer patients. |
|---|---|---|---|---|
| **Organ** | **Mice** | **Humans** | **Ref.** |
| | Healthy | Tumor-Bearing | Healthy | Cancer Patient |
| Peripheral blood | 5% | 60% | 0.13% | 2–4.25% | [41,42] |
| Spleen | 5% | 20–50% | 0.02% | 0.15% | [41,43,62,123] |
| Bone marrow | 15–20% | 55% | 14% | n.d. | [41,43,134] |
| Liver | 10% | 2–30% | 2.5% | 10% | [41,42,62] |
| Lymph node | 1% | 1% | n.d. | n.d. | [41,62] |
| Tumor | - | 2–10% | - | 10% | [41,42,62] |

n.d.—no data, - — not applicable.

6. Cancer-Induced Dysregulation of Erythropoiesis

The main cause of EPC expansion is the increase in EPO concentrations in response to anemia. However, the mechanisms of EPC induction by cancer are complex and rely on multiple components that dysregulate erythropoiesis (Figure 2), leading to ineffective erythropoiesis, characterized by erythroid differentiation arrest and increased apoptosis of erythroid cells, and is a feature of various diseases, including β-thalassemia [135]. Importantly, emerging evidence suggests that cancers not only induce potent EPC expansion, but also arrest their development at the earliest stages of differentiation. This leads to the suppression of immune response driven by EPCs, which are potent but physiologically transient immunosuppressors [92].
Figure 2. Mechanisms of erythropoiesis dysregulation in cancer. Expansion of early-stage EPCs is caused by (A) chronic erythropoietin (EPO) production. However, EPCs are unable to generate mature red blood cells (RBCs) due to increased apoptosis and differentiation arrest. EPCs apoptosis is triggered by (B) FasL/Fas and (C) TRAIL-TRAIL-R interaction between EPCs and cancer cells. Differentiation arrest of early-stage EPCs is caused by (D) transforming growth factor β (TGF-β), (E) iron restriction, (F) pro-inflammatory cytokines, and (G) cancer-secreted chemokines. Inhibited maturation is an effect of GATA1 degradation mediated by caspase-3 or p38 activation. (H) Bone marrow steady-state erythropoiesis is suppressed by inflammation and triggers stress erythropoiesis and expansion of EPCs in extramedullary sites.

6.1. Dysregulation of Hematopoietic Stem and Progenitor Cells Differentiation

The dysregulation of erythropoiesis by cancer begins at the first stage of hematopoiesis. Malignant hematological cells suppress hematopoietic stem and progenitor cells (HSPCs) in the bone marrow, limiting their differentiation and inducing a quiescent state. This suppression is mediated by various mechanisms, including SCF [51], TNF-α [136], arginase [137], TGF-β [138–140], and stanniocalcin 1 [141]. On the other side, HSPCs are enriched in the extramedullary sites, predominantly the spleen and the circulation of cancer patients, and are myeloid-biased to generate suppressive myeloid cells [142–146]. Cytokines and growth factors secreted by cancer cells and TME force hematopoiesis to the generation and maintenance of immunosuppressive cells that promote tumor growth [144]. Increased numbers of HSPCs in the circulation correlate with advanced tumor stage and decreased progression-free survival in cancer patients [142,146]. In cancer, TNF-α secreted by activated T-cells increases the proliferation of HSPCs and induces emergency myelopoiesis in the bone marrow [143]. Nonetheless, despite strong myeloid polarization, HSPCs in the extramedullary sites, including the spleen, also exhibit increased capacity to differentiate into BFU-E in tumor-bearing mice compared to healthy mice [144].
6.2. Disruption of Hematopoietic Stem and Progenitor Cells Niche

Cancer cells also directly impair HSPCs’ maintenance and differentiation by disrupting their niche, resulting in the loss of quiescence and stemness of HSPCs [51,147]. This phenomenon is the most prominent for hematological malignancies that primarily develop in the bone marrow and outcompete native HSPC niches [148]. Nonetheless, solid tumors were also reported to disrupt the HSPCs’ niche. Melanoma cells secrete vascular endothelial growth factor (VEGF), which reduces available vascular niches in bone marrow, promoting HSC mobilization [149]. Moreover, tumor-secreted exosomes educate bone marrow cells toward a pro-metastatic phenotype [150] and promote the production of pro-inflammatory cytokines by mesenchymal stem cells to support cancer cell growth while suppressing HSPCs [151].

6.3. Suppression of Erythroid Differentiation of Hematopoiesis Stem Cells

The differentiation of HSCs is skewed towards myelopoiesis by cancer. Thus, the potential of erythroid differentiation of HSCs is commonly suppressed, especially in the bone marrow. EPC precursors, MEPs, are the most suppressed progenitor cells in the bone marrow of mice with hematological malignancies [57,58,136,140,152–154]. In plasma cell myeloma, malignant cell infiltration correlates negatively with hemoglobin concentration, but not with leukocytes or platelet counts, which suggests the selective impairment of erythropoiesis by malignant cells [155]. This suppression is partially compensated by the increased proliferation of early-stage EPCs in cancer [57]. Moreover, erythroid progenitors are activated in extramedullary sites, including the spleen [154].

6.4. Chronic Erythropoietin Production

EPC expansion is triggered primarily by EPO, which promotes the survival, proliferation, and differentiation of EPCs [156]. EPO induces the expansion of highly proliferative early-stage EPCs [157]. Normally, this response is rapid and EPO concentration quickly decreases after the induction of EPC expansion, which results in RBC generation [158]. However, when the erythropoietic response is insufficient to rescue anemia, EPO is produced constantly. This results in the substantial expansion of early-stage EPCs in the bone marrow as well as in the extramedullary sites. In cancer, EPO is secreted predominantly in response to tissue hypoxia resulting from anemia.

Moreover, tumors may directly trigger the upregulation of EPO production in a vascular endothelial growth factor (VEGF)-dependent mechanism. Vascular endothelial growth factor (VEGF) is a growth factor produced by malignant and stromal cells in TME to induce neovascularization, vessel remodeling [159], and to modulate antitumor immune response [160]. VEGF concentration is substantially increased in the plasma of cancer patients [161]. Importantly, VEGF stimulates EPO secretion by splenic stromal cells expressing platelet-derived growth factor receptor β (PDGFR-β) [162]. Increased VEGF concentration in plasma leads to the increased reticulocyte index in the circulation and expansion of early-stage EPCs in the bone marrow and the spleen [163].

6.5. Induction of Erythroid Cell Apoptosis

Another mechanism of erythropoiesis dysregulation is the direct induction of erythroid cell apoptosis by cancer cells. Apoptosis induced by death receptor Fas (CD95) and Fas ligand (FasL, CD95L, or CD178) interaction is a critical negative regulatory axis of erythropoiesis [76,82]. These negative signals can be overcome by high EPO concentrations that promote EPC expansion during erythropoietic stress response [164].

Cancer cells secrete multiple factors that induce the expression of death receptors on EPCs, increasing their susceptibility to apoptosis [59,63]. Thus, EPCs from cancer patients have significantly upregulated Fas receptor [59,63]. Moreover, ligands for cell death receptors are commonly overexpressed by malignant cells [59,63,165]. The activation of death receptors triggers the activation of caspases that cleave GATA1 transcription factors, leading to the maturation arrest or apoptosis of EPCs [166]. Maturation arrest
caused by GATA1 degradation results in the accumulation of EPCs at the earliest stages of differentiation [155,166]. Moreover, GATA1 downregulation decreases the induction of anti-apoptotic proteins, including Bcl-xL and Bcl-2 [167,168]. The enhanced loss of erythroid precursors due to apoptosis leads to compensatory mechanisms and, consequently, higher percentages of early erythroblasts in the bone marrow of cancer patients [59].

Similar to FasL, malignant cells have an increased level of TNF-related apoptosis-inducing ligand (TRAIL) [59,63,169]. EPCs are characterized by the physiological expression of TRAIL receptors [170]. Their stimulation by TRAIL on cancer cells induces differentiation arrest caused by the activation of caspases and the induction of ERK1/ERK2 signaling [82,170,171].

### 6.6. Transforming Growth Factor β (TGF-β)

TGF-β is an important cytokine that promotes tumor growth and immune evasion [172]. Its concentration is substantially increased in the TME and serum of cancer patients [62,140,172–174]. Overactivation of the TGF-β pathway affects not only cells in TME, but also hematopoietic cells. Indeed, in cancer patients TGF-β signaling is the most dysregulated signaling pathway in HSPCs, which leads to impaired hematopoiesis, especially erythropoiesis [140]. In erythroid cells, TGF-β potently inhibits proliferation and self-renewal, but at a low concentration it may accelerate the differentiation of late-stage EPCs by promoting mitophagy [83,175–178].

TGF-β induces the maturation arrest of early-stage EPCs by noncanonical activation of p38, which in turn triggers GATA1 degradation [57,58,140]. Moreover, TGF-β activates SMAD2 and SMAD3 via the type III TGF-β receptor, which is transiently upregulated in early-stage EPCs [178]. Indeed, EPCs in tumor-bearing mice have overactivated SMAD2 and SMAD3 [62]. Accordingly, tumor-induced expansion of EPCs is substantially reduced in Smad3-deficient mice [62]. Moreover, EPC expansion may be prevented by the treatment with neutralizing antibody against TGF-β [62]. The ability to induce EPCs is decreased in mice bearing TGF-β-deficient tumor cells; however, not completely [62]. These findings confirm that TGF-β secreted by tumor cells and also by non-malignant cells in TME is a key factor inducing EPC expansion in cancer via SMAD signaling.

Another mechanism by which TGF-β impairs erythropoiesis involves IL-33, a member of the IL-1 superfamily of cytokines. Tumor-secreted TGF-β induces the expression of IL-33 in TME [179]. Indeed, an increased concentration of IL-33 has been reported in different types of cancer and often correlates with poor prognosis [180]. Notably, IL-33 potently inhibits the differentiation of EPCs at early stages by NF-κB activation and the inhibition of signaling pathways downstream of erythropoietin receptor (EPO-R) [181].

Other members of the TGF-β superfamily, including growth differentiation factor 11 (GDF11, also known as BMP11) and GDF15, have a similar role in the regulation of erythropoiesis. GDF11 induces the differentiation arrest of early-stage EPCs by the activation of SMAD2 and SMAD3 pathways, inhibiting terminal differentiation [182–184]. In myelodysplastic syndrome (MDS) patients, GDF11 serum concentration is negatively correlated with late erythropoiesis [185]. Erythropoiesis is also suppressed by GDF15, which modulates iron metabolism [186].

On the other side, some members of the BMP pathway, including BMP4, are crucial regulators of stress erythropoiesis and initiate the differentiation and expansion of EPCs, enabling erythropoietic response [128,187,188].

### 6.7. Iron Restriction

Iron is an important trace element required for many biological processes, including the heme synthesis [78,189]. Thus, its metabolism is regulated by multiple proteins including iron-transporting transferrin, iron-storing ferritin, and ferroportin responsible for iron export from the cell [190]. Absolute iron deficiency is detected in over 40% of cancer patients [191]. Notably, iron restriction selectively impairs erythroid cell differentiation, but not granulocytic nor megakaryocytic progenitors [61,192–194]. Iron is a metabolic
checkpoint that restrains the expansion of EPCs triggered by EPO in the case of insufficient iron availability. Iron restriction downregulates the crucial control element of the EPO receptor, Scribble, preventing further EPC maturation [61]. Moreover, iron control of EPC differentiation is mediated by an aconitase-associated regulatory pathway that compromises heme production and modulates EPO signaling [194]. This results in profound changes in the gene expression profile, including the downregulation of GATA1 and its target genes, leading to the impairment of EPC maturation with the differentiation arrest of early EPCs [192–195].

EPCs can obtain and concentrate iron with exceptional efficacy [196]. Nonetheless, cancer cells and nonmalignant cells in TME are also characterized by increased iron metabolism [197,198]. Cancer cells overexpress CD71 and compete with the EPCs for transferrin-bound iron [199]. Moreover, cells in TME, especially macrophages, accumulate iron, leading to its sequestration from EPCs and exaggerating iron deficiency [165].

6.8. Pro-Inflammatory Cytokine-Driven Erythropoiesis Impairment

Anemia of inflammation (also referred to as anemia of chronic disease) is associated with systemic inflammation, which is one of the hallmarks of cancer and is primarily caused by altered iron distribution [200,201]. Inflammation activates the inflammasome, which triggers enzymatic activation of caspases [202]. Inflammasome assembly in HSPCs leads to the GATA1 cleavage by caspase-1, which favors myelopoiesis over erythropoiesis and suppresses terminal erythropoiesis, leading to the maturation arrest of EPCs [203]. In mice expressing active \( \text{Kras}^{G12D} \), the activation of inflammasome leads to myeloproliferation and anemia with a compensatory expansion of EPCs in peripheral blood [204]. In this model, anemia as well as EPC expansion are reduced after pharmacological inflammasome inhibition [204].

Chronic inflammation inhibits the late-stage differentiation of EPCs, leading to the maturation arrest of the early-stage EPCs, which is mediated by various cytokines [205]. One of the critical mediators of inflammation is interferon \( \gamma \) (IFN-\( \gamma \)) [206], which also potently impairs erythropoiesis, leading to anemia [207]. Erythroid cells stimulated with IFN-\( \gamma \) have increased levels of pro-apoptotic caspases, induced differentiation arrest, and triggered apoptosis [208,209]. Moreover, IFN-\( \gamma \) upregulates the expression of Fas on EPCs, increasing their susceptibility to apoptosis in vivo [210]. Additionally, IFN-\( \gamma \) induces the expression of a key regulator of myeloid differentiation, PU.1, in EPCs [207]. During physiological erythropoiesis, the expression of PU.1 is downregulated due to the inhibitory effects on GATA1 functions and erythroid cell differentiation [211–213]. Thus, chronic IFN-\( \gamma \) production results in decreased erythropoietic activity in the bone marrow, but increased myelopoietic activity [207]. Moreover, IFN-\( \gamma \) reduces RBC life span and increases macrophage erythropagocytosis, aggravating anemia and stimulating EPC expansion [207].

Similar suppressive effects on erythropoiesis have been described for another pro-inflammatory cytokine, TNF-\( \alpha \). Cancer patients are characterized by the chronic production of TNF-\( \alpha \), which promotes immune escape and tumor progression [214]. TNF-\( \alpha \) induces the maturation arrest of early-stage EPCs and promotes their apoptosis [82,215–218]. This effect is mediated by the p55 TNF receptor and the activation of caspases [82,215]. TNF-\( \alpha \) also upregulates p38 MAPK in EPCs, which phosphorylates acetylated GATA1, promoting its degradation [219,220]. Moreover, TNF-\( \alpha \) upregulates PU.1 and GATA2 in HSPCs, which antagonize erythroid cell differentiation [221].

Likewise, the maturation arrest of EPCs at early stages and the inhibition of EPC proliferation are also triggered by other proinflammatory cytokines that are overexpressed in cancer, including IL-1 [222], IL-6 [223] or IL-12 [224].

6.9. Cancer-Secreted Chemokines

Erythropoiesis is also influenced by dysregulated chemokine profiles in the bone marrow plasma and serum of cancer patients. One of these chemokines is CCL3, which
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is upregulated in the majority of patients with hematopoietic malignancies [57,58] and a subset of patients with solid tumors [225]. CCL3 suppresses erythroid differentiation by p38 activation via CCR1, and this leads to the degradation of GATA1 [57,58,226]. On the other side, chemokines may also promote erythropoiesis by recruiting monocyte-derived macrophages to create erythroblastic islands in the extramedullary sites [227].

6.10. Induction of Extramedullary Stress Erythropoiesis

Suppressed bone marrow steady-state erythropoiesis is a hallmark of inflammation and is caused by the production of pro-inflammatory cytokines and iron sequestration [130,207,228,229]. Suppression steady-state erythropoiesis is often observed in patients with hematological malignancies [136,140,152,153] and solid tumors [230]. As a consequence, stress-erythropoiesis is activated in extramedullary sites to maintain erythroid homeostasis [129]. EPO secreted in response to anemia promotes the formation of erythroblastic islands in the spleen followed by the extensive proliferation of erythroid cells [228,231]. Multiple inflammatory cytokines that suppress erythropoiesis in the bone marrow simultaneously induce stress erythropoiesis. This effect was reported for IFN-γ [207], TNF-α [229], IL-1β [229,232], IL-6 [223], and G-CSF [233]. Notably, extramedullary stress erythropoiesis may be also induced by other factors, including ultraviolet B (UVB) exposure, tumor-promoting environmental stress [43], and chronic stress [234,235], which often accompany cancer.

Importantly, EPCs in extramedullary sites may still exhibit differentiation arrest that results in the enrichment of early-stage EPCs [207]. Thus, early-stage EPC fraction is increased in the spleen of tumor-bearing mice compared with acute anemic mice that also have induced stress erythropoiesis [41].

6.11. Chemotherapy-Induced Impairment of Erythropoiesis

The myelosuppressive effects of chemotherapy are another cause of anemia in cancer [166]. Importantly, early-stage EPCs are especially sensitive to the cytotoxic effects of chemotherapeutic agents, while late-stage EPCs are more resistant [236]. EPC apoptosis triggered by chemotherapy is induced by caspase activation and can be prevented by the SCF-mediated up-regulation of anti-apoptotic proteins Bcl-2 and Bcl-XL [236]. Therefore, it was suggested that SCF may be used in the supportive therapy of chemotherapy-treated cancer patients [237]. This approach may diminish the development of anemia, which would cause the extensive expansion of EPCs after treatment.

7. Modulation of EPCs to Inhibit Their Tumor-Promoting Effects

The development of strategies that modulate the immune response in cancer patients is of great clinical interest. The modulation of immunosuppressive and tumor growth-promoting EPC mechanisms is a promising approach to diminish their negative role. Moreover, treating anemia to prevent EPC expansion as well as targeting ineffective erythropoiesis may causally decrease the tumor-promoting effects of EPCs.

7.1. Modulation of EPCs Immunosuppressive Mechanisms

7.1.1. Reactive Oxygen Species

The production of ROS by EPCs is a key mechanism of immune suppression as ROS scavengers substantially rescue T-cell function suppressed by both murine and human EPCs [41,42,116]. Several antioxidant-based therapies were demonstrated to have potent antitumor effects in preclinical studies [238]. However, further studies revealed that antioxidants may accelerate tumor progression and promote metastasis [239,240]. Therefore, current studies focus on increasing rather than decreasing ROS levels in TME due to increased vulnerability to oxidative stress-induced apoptosis [241]. Indeed, ROS-generating agents or inhibitors of antioxidant systems are efficient in preclinical studies; however, they are without satisfactory results in clinical trials [242]. Thus, more research is required to determine the clinical utility of ROS-based therapies in cancer.
7.1.2. IL-10

IL-10 was considered for many years as a potent anti-inflammatory cytokine. Accordingly, EPCs were found to secrete IL-10, which suppresses T-cells [42]. However, many studies in this field demonstrated that its role in cancer is more complex than initially envisioned [243–246]. Intriguingly, IL-10-based therapy, including pegylated IL-10 (Pegilodecakin), is much more efficient than therapies neutralizing IL-10 effects [246–248].

7.1.3. PD-L1/PD-1 Axis

Targeting immune checkpoints has revolutionized clinical oncology. Monoclonal antibodies targeting PD-L1 or PD-1 reverse the inhibitory signals triggered by the PD-L1/PD-1 axis and enhance antitumor immune response [249–251]. PD-L1 is also expressed by murine and human tumor-induced EPCs [43]. Interestingly, the expression of PD-L1 is higher in stress erythropoiesis EPCs compared to steady-state EPCs in the bone marrow, and it reaches the highest levels in tumor-infiltrating EPCs [43]. Although an exact role of the PD-L1/PD-1 axis in the EPC-mediated suppression of immune response was not assessed, it seems that immune checkpoint inhibitors may at least partially diminish their tumor-promoting effects.

7.1.4. TGF-β

The production of TGF-β in the TME is crucial to induce and maintain its immunosuppressive character [172]. TGF-β is produced by various types of cells, including EPCs [42]. The inhibition of SMAD signaling induced by TGF-β rescues T-cell proliferation and the production of IFN-γ suppressed by EPCs [42]. Therefore, modulating TGF-β signaling is a promising strategy to attenuate immune evasion induced by tumor-associated cells, including EPCs. Indeed, several anti-TGF-β-based immunotherapies were shown to be effective in preclinical studies, especially in combination with immune checkpoint inhibitors [252–255]. Therefore, targeting TGF-β signaling is a promising approach to suppress the tumor-promoting effects of EPCs.

7.2. Anti-Artemin Therapy

Late-stage EPCs promote tumor growth and invasiveness via the secretion of artemin [62,123]. Anti-artemin neutralizing antibody inhibits tumor growth and increases the survival of tumor-bearing mice [62]. Anti-artemin therapy is also currently tested for the treatment of cystitis-induced bladder hyperalgesia [256]. However, the clinical utility of targeting artemin or its signaling as the modulation of tumor growth-promoting effects of EPCs is unknown.

7.3. Treating Anemia to Prevent EPC Expansion

Since the modulation of EPCs’ tumor-promoting mechanisms is rather ineffective, a decrease in EPC expansion and the induction of their differentiation is a promising strategy. The correction of anemia in cancer patients is one of the strategies to prevent EPC expansion. Most anemic patients have iron deficiency (ID) [191]; therefore, the determination of iron status and treatment is recommended for cancer patients according to the guidelines [66,257,258]. Current European Society for Medical Oncology (ESMO) guidelines of anemia management in cancer patients are presented in Figure 3.
Figure 3. Management of anemia in cancer patients according to ESMO guidelines [258]. Additionally to TSAT and SF, the percentage of hypochromic cells (%HYPO) > 5% and reticulocytes hemoglobin content (CHr) < 28 pg can be used to determine impaired iron status. ID can be treated with oral iron only if ferritin < 30 ng/mL and CRP < 5 mg/L. CRP—C-reactive protein, ESA—erythropoiesis-stimulating agent, Hb—hemoglobin, i.v.—intravenous, ID—iron deficiency, RBCs—red blood cells, SF—serum ferritin, TSAT—transferrin saturation.

Impaired iron status can be diagnosed by total iron-binding capacity (TIBC), transferrin saturation (TSAT), or serum ferritin (SF) levels tests. TSAT enables the determination of iron status available for erythropoiesis, and its low levels together with high SF (>100 ng/mL) suggest functional iron deficiency (FID) [66]. Iron should be supplemented intravenously or orally for patients with low ferritin and without anemia of inflammation (CRP < 5 mg/L) [66].

However, anemia in cancer is not commonly caused by absolute ID, but rather results from iron sequestration (functional ID) [66,200,201]. In this group of patients, iron-replenishing strategies may not be effective. Therefore, targeting iron metabolisms with hepcidin antagonists to modulate the hepcidin-ferroportin axis is a promising treatment option [66,259]. Moreover, several novel therapies are currently under investigation for cancer-associated anemia, including ascorbic acid, androgens, BMP2 and BMP6 antagonists, as well as activin traps [66].

7.4. Targeting Ineffective Erythropoiesis to Decrease EPC Expansion

Modulating EPC expansion by promoting EPC differentiation is a novel therapeutic strategy to diminish the tumor-promoting effects of EPCs (Figure 4).
Figure 4. Targeting EPC expansion as a novel therapeutic strategy. Cancer-induced dysregulation of erythropoiesis resulting in the differentiation arrest of EPCs and their expansion may be diminished by different agents. Inhibitors of (1) transforming growth factor β (TGF-β) and (2) BMP signaling rescue maturation arrest. (3) Caspases inhibitors inhibit GATA1 cleavage triggered by the inflammasome. (4) P38 inhibitors promote differentiation and decrease the apoptosis of EPCs. (5) JAK inhibitors decrease activation of EPO-induced signaling, which decreases EPCs expansion. (6) mTOR inhibitors and inducers of FoxO3 promote differentiation of early-stage EPCs. (7) Increasing serotonin (5-HT) concentration with either selective serotonin reuptake inhibitors (SSRIs) or inhibitors of the kynurenine metabolism pathway promotes differentiation of EPCs. (8) Enasidenib, an inhibitor of mutated IDH2 promotes terminal differentiation of EPCs.

TGF-β is a key negative regulator of erythropoiesis, which triggers differentiation arrest and promotes the expansion of EPCs [62]. TGF-β inhibitors stimulate EPC differentiation in vitro [178,260]. In murine models, the anti-TGF-β antibody inhibits tumor growth and prevents the expansion of CD45− EPCs [62]. Moreover, SMAD inhibitors rescue T-cell proliferation and IFN-γ production suppressed by EPCs [42]. Therefore, the modulation of TGF-β and SMAD2/3 signaling is a promising approach to promote erythroid cell maturation and to diminish their suppressive effects [261], despite the lack of potent antitumor effects of monotherapy in clinical trials [262].

Myelodysplastic syndromes (MDS) are characterized by the impairment of erythroid cell differentiation with maturation arrest at the early stage [263,264]. In murine MDS models, TGF-β superfamily ligand-trapping fusion protein ACE-536 promotes erythroid
maturation by binding GDF11, inhibiting SMAD2/3 signaling, and promoting late-stage erythropoiesis, reducing rescue anemia [182]. Similar effects are exerted by the compound ACE-011, which promotes terminal erythropoiesis and prevents EPC expansion in β-thalassemia [184]. In clinical trials, luspatercept (ACE-536) and sotatercept (ACE-011) reduced the severity of anemia in patients with MDS and β-thalassemia [265–267]. Clinical trials of sotatercept in cancer patients showed that it may also be effective for the treatment of chemotherapy-induced anemia [268].

Moreover, it was suggested that targeting BMP signaling may be beneficial for anemia of inflammation [269]. The inhibition of BMP downregulates IL-6 signaling and decreases hepcidin levels, resulting in the restoration of erythropoiesis suppressed by inflammation [270,271].

Caspase-1 activation by inflammasome is one of the mechanisms skewing the differentiation of HSPCs toward myeloid cells [203]. Thus, the inhibition of caspase-1 results in the upregulation of GATA1 and the rescue of inflammation-induced anemia [203]. Moreover, caspase inhibitors trigger the differentiation of EPCs after the induction of maturation arrest by FasL or TNF-α [82]. Several caspase-1 inhibitors are available, including a potent and selective inhibitor, VX-765 [272]. Therefore, it is of great interest to evaluate the effects of caspase inhibitors on EPC expansion and differentiation in cancer.

P38 MAPK is an important pathway regulating erythropoiesis. P38 is activated by multiple inflammatory signals and restrains EPC differentiation by GATA1 degradation [220] and by suppressing the silencing of Bim, which triggers apoptosis [273]. Moreover, p38 functions as an oncogenic kinase with a complex role in cancer [274]. Therefore, p38 inhibition may simultaneously have an antitumor effect and promote EPC maturation. Notably, p38 inhibition enhances EPC maturation in an EPO-independent manner [273], but also decreases the production of endogenous EPO under stress conditions [275], suggesting that p38 inhibitor therapy should probably be combined with ESAs.

In erythroid cells, EPO triggers EPO-R and association with cytoplasmic Janus kinase 2 (JAK2), a crucial signal transducer [276]. The overactivation of JAK2, most commonly caused by V617F mutation, is associated with myeloproliferative neoplasms, including polycythemia vera [277]. Nonetheless, increased JAK2 activity may also be caused by high levels of EPO caused by anemia and chronic hypoxia, a state observed in β-thalassemia and cancer. In β-thalassemia, JAK2 inhibitors decrease ineffective erythropoiesis, prevent the expansion of EPCs, and reduce splenomegaly [278]. JAK2 inhibitors, including ruxolitinib and fedratinib, are approved for the treatment of patients with MPNs [279]. Moreover, targeting JAKs is a promising therapeutic strategy for the treatment of different types of cancer [280]. Whether JAKs inhibitors may decrease the expansion of EPCs in cancer patients remains unknown.

The mechanistic target of rapamycin (mTOR) is a central protein kinase orchestrating cell growth, metabolism, and immune response. Thus, mTOR is widely tested in clinical trials as a target for cancer therapy [281]. Importantly, mTOR inhibition rescues EPC differentiation under ineffective erythropoiesis by inducing the cell cycle exit of early-stage EPCs [282]. Moreover, mTOR inhibition in EPCs may be triggered by Forkhead-box-class-O3 (FoxO3) [282]. The activation of FoxO3 by resveratrol induces early erythroid maturation and decreases their proliferation, resulting in a reduction in ineffective erythropoiesis [283].

Erythropoiesis is also regulated by serotonin (5-HT) [284,285]. Dysregulated tryptophan metabolism with an enhanced kynurenine pathway is common in cancer, and is increasingly being recognized as a viable metabolic pathway regulating immune response [286]. A skew towards the kynurenine pathway leads to decreased serotonin (5-HT) concentrations, resulting in the impaired differentiation and decreased survival of EPCs [284,285]. The upregulation of 5-HT triggered by EPO is crucial to protect EPCs from apoptosis at the CFU-E-to-proerythroblast transition checkpoint [284]. Pharmacological increase in 5-HT with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), rescues
anemia [284]. Therefore, targeting the 5-HT axis in EPCs with either SSRI or kynurenine
pathway inhibitors may diminish the tumor-promoting role of immature erythroid cells.

Enasidenib is an Food and Drug Administration (FDA)-approved, first-in-class prefer-
ential inhibitor of mutated isocitrate dehydrogenase 2 (IDH2) that promotes the differenti-
ation of acute myeloid leukemia blasts [287]. Interestingly, enasidenib was found to act
independently of IDH2 on EPCs. Enasidenib potently promotes erythroid differentiation
through the modulation of protoporphyrin IX (PPIX) accumulation and hemoglobin pro-
duction in late-stage EPCs [288]. As a result, increased hemoglobin concentration and RBC
transfusion independence were reported for enasidenib-treated patients [287,289].

Some studies suggested that natural compounds may decrease EPC expansion. Dang-
guibuxue decoction (DGBX), a traditional Chinese medicine, abolishes EPC accumulation,
promotes their differentiation, and rescues anemia, leading to the activation of anti-tumor
immune response and a decrease in tumor growth [290]. Moreover, a recent study revealed
that vitamin C has a critical role in the regulation of late-stage erythropoiesis and is able to
rescue ineffective erythropoiesis [291].

7.5. Splenectomy

In cancer, the spleen becomes a central organ of extramedullary hematopoiesis, re-
sponsible for the generation of suppressive cells including EPCs and myeloid cells [292].
Therefore, it was suggested that splenectomy could be beneficial for cancer patients. In
preclinical models, splenectomy inhibits tumor growth and prolongs the survival of tumor-
bearing mice [62]. It also abolishes the induction of EPC expansion in extramedullary
sites [62]. Similarly, splenectomy leads to the depletion of MDSCs, enhancing the activation
of antitumor immunity [293]. Intriguingly, splenectomy before tumor inoculation or during
tumor progression attenuates the decrease in RBC count and hemoglobin concentration,
alleviating anemia [62].

However, clinical data are much less promising. Randomized trials showed that
splenectomy in cancer patients not only has no advantages, but is also associated with
increased perioperative morbidity [294–296]. Therefore, more preclinical and clinical
studies are required to evaluate the effects of splenectomy in cancer.

8. Clinical Consequences of Tumor-Induced Anemia and EPC Expansion

Anemia is very common in cancer patients. Its prevalence differs from 30–90% de-
pending on the type of cancer as well as the diagnostic criteria. It substantially decreases
the quality of life of cancer patients [296,297]. Moreover, anemia is associated with shorter
survival for patients with different types of cancer and a 65% overall increase in the risk
of mortality compared to non-anemic cancer patients [191,298,299]. Importantly, severe
anemia is associated with hypoxia in the TME of both primary and metastatic tumors [132],
which is a known driver of aggressive tumor phenotype [300].

Clinical outcomes of EPC expansion are still unclear. In cancer patients, EPC expa-
sion is the most prominent in individuals with moderate or severe anemia [41]. Moreover,
the expansion of CD45+ EPCs correlates with a higher EBV load and suppressed T-cell
response against the major antigenic EBV proteins, LMP2 and EBNA1 [41]. A recent study
also demonstrated that CD45− EPCs may have clinical significance. In PDAC patients,
the counts of CD45− EPCs in the spleen are increased compared with noncancerous pancreatic
tumors or benign pancreatic masses [123]. High CD45− EPC counts predicted poor progno-
sis and were associated with larger tumor size and lymph node metastases [123]. Moreover,
increased serum artemin concentrations, as well as increased expression of its receptors,
correlate with poor prognosis in cancer patients [62,123]. Collectively, these observations
demonstrate that in cancer patients, early-stage CD45+ EPCs may suppress the immune
response, and late-stage CD45− EPCs may promote tumor growth by the secretion of
artemin. Nonetheless, more research is required to accurately dissect the clinical role of
EPCs in cancer patients.
9. Conclusions

In recent years, we have expanded our knowledge regarding the mechanisms of tumor evasion induced by dysregulation in hematopoiesis. The initial assumption that cancer only significantly regulates myelopoiesis turned out to be an oversimplification. Emerging evidence demonstrates that harnessing erythroid lineage cells together with megakaryocytes and platelets [301,302] is critical for cancer progression and immune evasion. EPCs promote tumor growth by either suppressing anti-tumor immune response or secreting growth factors, depending on the developmental stage. EPCs share many similarities with well-described suppressive cells of the immune system and use the same mechanisms to regulate the immune response.

However, several issues remain unclear and need to be investigated. First, despite differences in the expression of the immunomodulatory molecules [43], factors regulating the immunosuppressive properties of EPCs are unknown. Presumably, tumor-secreted cytokines and TME may potentiate the tumor-promoting role of EPCs. Moreover, it remains elusive whether and, if so, what factors promote the recruitment of EPCs to TME. Additionally, it is not known what are the interactions between EPCs and other cells in TME, including MDSCs, TAMs or NK cells, and comprehensive studies on the role of EPCs in TME are currently limited by technological advances. Recently, transcriptional profiling at a single-cell level has revolutionized our understanding of the complexity of cell interactions in TME, and indicates further research directions [303]. However, most of the protocols involve extensive hypotonic lysis of red blood cells [304], which drastically reduces the number of EPCs [305], excluding them from analyses of TME networks. Therefore, there is a great need to define the whole landscape of TME that includes EPCs.

Future research should focus on the comprehensive characterization of immunomodulatory mechanisms of EPCs and their regulation to better understand their function in tumor immune evasion and to enable targeting them in immunotherapy. It remains unknown whether EPCs in cancer may induce Treg differentiation, similar to their neonatal counterparts [87]. Similarly, regardless of a well-established role of EPCs in neonates [86,94], the regulation of myeloid cell response by EPCs in cancer is unknown. Moreover, erythroid cells were reported to produce IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α [306]; however, their role in immune regulation by EPCs remains unknown.

It needs to be determined whether and how EPCs contribute to the clinical outcome of cancer patients undergoing various types of treatment, including immunotherapy. It was reported that EPCs may contribute to the drug resistance of cancer cells [116]. Therefore, targeting EPCs or their effector mechanisms in combination with other therapies may improve therapeutic effectiveness. Finally, the development and clinical testing of agents that could rescue erythroid maturation under cancer-induced EPC differentiation arrest are of great interest. Until then, anemia treatment is the best strategy to reduce EPC expansion and differentiation arrest, as well as to minimalize the tumor-promoting role of EPCs and to improve the survival and quality of life of cancer patients.

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