Semaphorin 6 Family Proteins Drive Cancer Progression

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According to recent evidence, some groups of semaphorins (SEMAs) have been associated with cancer progression. These proteins are able to modulate the cellular signaling of particular receptor tyrosine kinases (RTKs) via the stimulation of SEMA-specific coreceptors, namely plexins (plexin-A, -B, -C, -D) and neuropilins (Np1, Np2), which share common domains with RTKs, leading to the coactivation of the latter receptors. MET, ERBB2, VEGFR2, PFGFR, and EGFR, among others, represent acknowledged targets of semaphorins that are often associated with tumor progression or poor prognosis. In particular, higher expression of SEMA6 family proteins in cancer cells and stromal cells of the cancer niche is often associated with enhanced tumor angiogenesis, metastasis, and resistance to anticancer therapy. Notably, high SEMA6 expression in malignant tumor cells such as melanoma, pleural mesothelioma, gastric cancer, lung adenocarcinoma, and glioblastoma may serve as a prognostic biomarker of tumor progression. To date, very few studies have focused on the mechanisms of transmembrane SEMA6-driven tumor progression and its underlying interplay with RTKs within the tumor microenvironment. The growing evidence is presented in the literature on the complex and shaping role of SEMA6 family proteins in cancer responsiveness to environmental stimuli.

SEMA6 plexins RTKs VEGF VEGFR1/2 tumor dendritic cells

1. Semaphorin 6A

Semaphorin 6A is the best-known subclass of semaphorins in the SEMA6 family. Its first documented role was in axon guidance and as an axon growth factor in the development of the central and peripheral nervous systems [1][2][3]. However, ongoing studies and progress in advanced molecular methods have unveiled multiple novel processes in oncogenesis that engage SEMA6 proteins. Angiogenesis and neovascularization are processes by which a tumor gains an uninterrupted flow of nutrients, both of which are crucial for the progression of the disease. The development of new vessels within the tumor niche is stimulated by the interaction of vascular endothelial growth factor (VEGF) with its receptors, vascular endothelial growth factor receptor 1 (VEGFR1) and vascular endothelial growth factor receptor 2 (VEGFR2). Interestingly, VEGF signaling has been found to be impaired by SEMA 6 deficiency [4]. In a study by Segarra et al., silencing of SEMA6A led to significant downregulation of VEGFR2 mRNA and protein expression in primary endothelial cells (HUVECs). Furthermore, a decrease in VEGFR2 levels was found to be critical for endothelial cell survival, and this process could not be compensated by exogenous VEGF. These observations were confirmed in mouse models deprived of SEMA6A. Complementary experiments using SEMA6A-null mice with reduced VEGFR2 expression showed impaired laser-induced choroidal

angiogenesis as well as compromised angiogenesis in melanoma and lung tumors in these mice. Finally, disrupted VEGF signaling and angiogenesis translated into a smaller tumor volume compared to mice with normal SEMA6A expression [4]. Collectively, control of VEGFR2 expression via SEMA6A expression impairment may be regarded as a promising new neoadjuvant modality to restrict tumor vascularization. Interestingly, a similar inhibitory effect on the vascularization of tumors in mice was achieved after the treatment of animals with SEMA6A-1 soluble extracellular domain (SEMA-ECD). Matrigel isolated from mice treated with SEMA-ECD at a rate of 1 mg/kg had a significantly lower density of developed blood vessels. Moreover, those vessels were over 2-fold shorter, and their branching points were markedly reduced. These results implied that SEMA-ECD inhibited neovascularization in tumor cells by arresting VEGF-driven Src, FAK, and ERK kinase signaling (**Figure 1**). This fact is certainly important in the context of therapeutic anticancer modalities and warrants further investigation in the future [5].

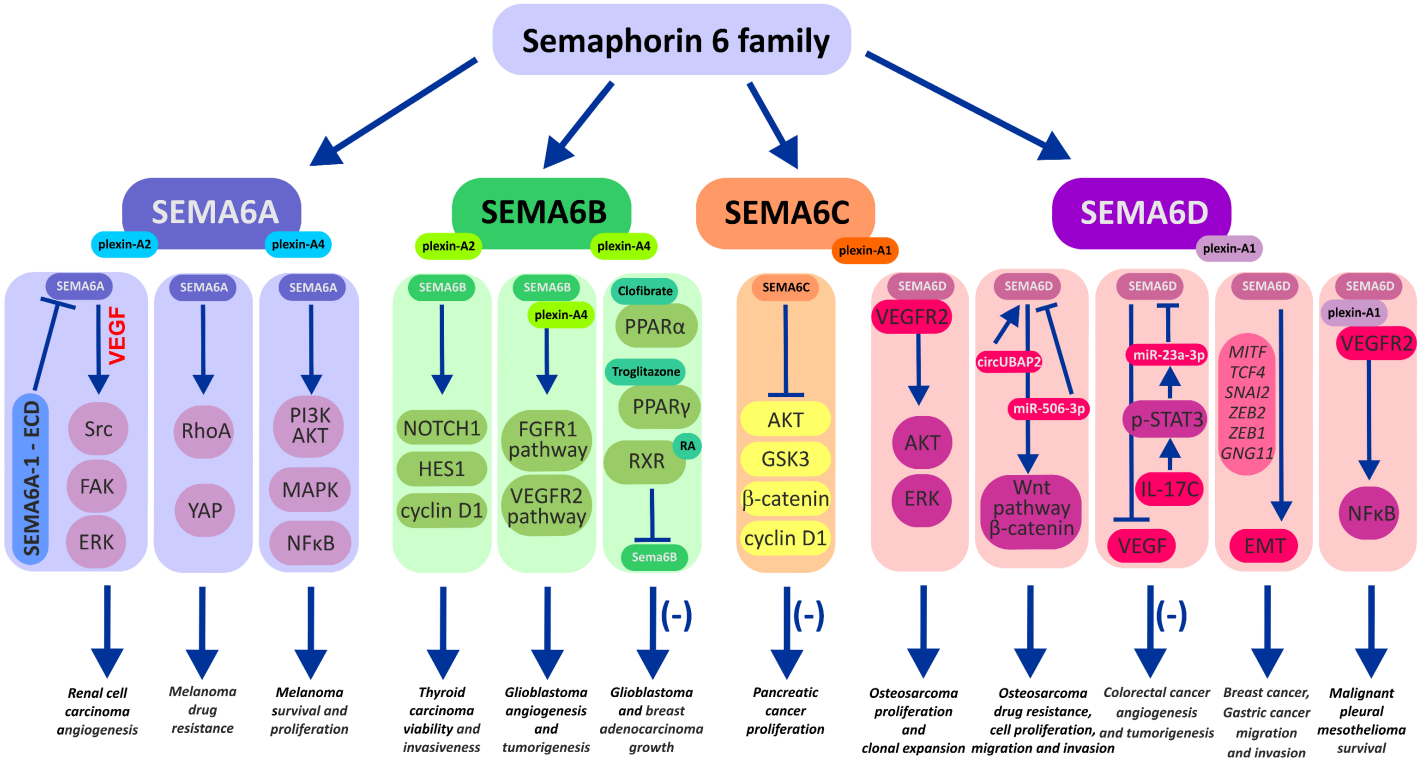

Figure 1. Illustration of semaphorin 6A–D signaling pathways and their interaction with the cellular molecular network in cancer cells. Activation of semaphorin signaling either via the “forward” (via plexins) or “reverse” mode by engaging RTK signaling is often associated with enhanced tumor angiogenesis, metastasis, and resistance to anticancer therapy. Pharmacological intervention (clofibrate, troglitazone, retinoic acid (RA)), molecular downregulation of SEMA6 activity (by dominant negative SEMA6A-1 soluble extracellular domain, SEMA-ECD), downregulation of protein expression (miRNA), or even upregulation of SEMA6 expression (SEMA6C) may be beneficial as adjuvants along with anticancer therapeutics. VEGF: vascular endothelial growth factor; VEGFR1/2: vascular endothelial growth factor receptor 1/2; Src: Src kinase; FAK: focal adhesion kinase; ERK: extracellular signal-regulated kinase; RhoA: transforming protein RhoA; YAP: Yes-associated protein; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; MAPK: mitogen-activated protein kinase; NfkB: nuclear factor kappa-light-chain-enhancer of activated B cells; NOTCH1: neurogenic locus notch homolog protein 1; HES1: transcription factor

HES1; FGFR1: fibroblast growth factor receptor 1; PPARα: peroxisome proliferator-activated receptor α; PPARγ: peroxisome proliferator-activated receptor γ; RXR: retinoid X receptor; GSK3: glycogen synthase kinase 3; Wnt: Wnt signaling pathway; STAT3: signal transducer and activator of transcription 3; EMT: epithelial to mesenchymal transition.

In most cancer types, upregulation of SEMA6A levels is associated with an overall poorer prognosis, increased invasiveness, and inhibition of apoptosis of mutated cells. These observations were confirmed in malignant glioma [6], gastric cancer [7], oral carcinoma [8], hepatocellular carcinoma [9][10], renal cell carcinoma [11], and melanoma [12][13][14], where SEMA6A activates pro-survival and pro-proliferative cellular signaling, including the PI3K/AKT, MAPK, and NFkB pathways [12]. In all these examples, the SEMA6A protein could be employed as a potential diagnostic biomarker and/or therapeutic target.

In contrast, low SEMA6A levels correlated with a worse prognosis for particular types of cancers. Such evidence was found for glioblastoma [15] and lung cancer [16]. Upregulation of SEMA6A levels in mice with lung cancer resulted in lower tumor volume. The apoptosis rate of cancer cells was increased, mainly through the FADD-associated apoptosis pathway [17]. In human lung cancer lines, overexpression of SEMA6A resulted in decreased migration due to the activation of the nuclear factor erythroid 2 p45-related factor 2 (NRF2)/heme oxygenase-1 (HMOX) axis, which translated into increased overall survival (OS) and a decreased recurrence rate [16]. In addition, SEMA6A level was found to be higher in nonsmoking lung cancer patients; thus, it could serve as a good biomarker for this narrow group of patients [18]. A similar association with lung cancer among nonsmokers was reported for SEMA5A; however, poor survival among nonsmoking women with NSCLC was related to transcriptional and translational downregulation of SEMA5A in cancer tissue [19].

Finally, there is emerging evidence indicating that SEMA6A can drive the drug resistance of cancer cells through a remodeling of their cytoskeletons. SEMA6A was found to form complexes with beta-actin in the cytoskeleton and to possibly be involved in the modulation of tubulin isotype composition and thus microtubule dynamics in human ovarian cancer cells [20]. Using quantitative PCR, Prislei and colleagues have shown that beta-tubulin III (TUBB3) overexpression/silencing correlates with SEMA6A expression in ovarian A2780 cancer cells. More importantly, the levels of SEMA6A were also higher in drug-resistant and BRAF-mutant melanoma [12]. In their study, Loria and colleagues observed that SEMA6A is partially engaged in the control of actin cytoskeleton remodeling of BRAF-mutated melanoma, which drives their fast rate of proliferation and survival. Moreover, the inhibition of BRAF and MEK kinases by combined dabrafenib + trametinib treatment of BRAF-mutated melanoma led to the activation of the SEMA6A/RhoA/YAP pathway, which resulted in the remodeling of the cytoskeleton and a reduction in targeted therapy efficiency. In particular, YAP is perceived as an oncogenic factor conferring cancer cell stemness, drug resistance, and metastasis associated with cellular cytoskeletal tension and cell-autonomous sensing and responsiveness to the stiffness of the tumor niche [21]. Finally, by unchaining BRAF-mutant melanoma from the stimulatory effects of cancer-associated fibroblasts via the depletion of SEMA6A and its related switched-off pro-survival (PI3K/AKT) and pro-proliferative (MAPK, NFκB) pathways, the authors were able to rescue the efficiency of BRAF/MEK inhibition against melanoma. In other words, these findings reveal SEMA6A as a new potential targetable protein to reverse drug resistance phenomena in cancer.

2. Semaphorin 6B

SEMA6B is a transmembrane protein which cooperates with the plexin-A4 receptor and plays a vital role in neuron organization via axon guidance [22][23], the development of hippocampal mossy fibers [24], and axon outgrowth inhibition in lesioned nerve tissue [25]. Moreover, *SEMA6B* gene mutations are a leading factor in the development of SEMA6B-related progressive myoclonic epilepsy (PME-11) [26][27][28][29][30][31][32].

Under pathological conditions, SEMA6B could be involved in thyroid cancer development. SEMA6B levels were found to be upregulated in these malignant tissues [33][34]. While enhanced SEMA6B improved cancer cell viability and invasiveness through the modulation of the NOTCH pathway, silencing of SEMA6B made cancer cells less viable and invasive. Taken together, these results showed SEMA6B is a potential drug target in the prevention and treatment of thyroid gland cancer [33]. Another study aimed at building a prognostic prediction model of thyroid carcinoma employed the pattern of *SEMA6B* gene expression in these malignant tissues [34]. In a *SEMA6B* gene model, upregulation of *SEMA6B* expression resulted in an overall poorer prognosis for patients. The study implied that *SEMA6B* expression, along with other genes tested (*PPBP*, *GCCR*), could be used as a prognostic marker in thyroid carcinoma.

SEMA6B levels were also found to be indicative of prognosis in breast cancer, the most diagnosed cancer among women. A study by Kuznetsova et al. demonstrated an abnormal methylation rate (38%) of the CpG islands in the *SEMA6B* gene, along with a frequent downregulation of SEMA6B expression in 44% of breast tumor samples tested (assayed by real-time PCR) [35]. Contrarily, methylation of the promoter region of *SEMA6B* was not observed in cultured breast cancer-derived cell lines MCF7 and T47D [35]. Interestingly, the potential role of a specific isoform, SEMA6Ba, in mammary tumorigenesis has been proposed; however, more in-depth studies are needed [36]. In contrast to breast cancer, high expression of *SEMA6B* was assessed in other types of tumors: gastric [37], gallbladder [38], and colorectal [39] cancers. Upregulation of *SEMA6B* was frequently associated with increased invasiveness, metastasis, and migration of cancer cells and worse overall prognosis. As expected, silencing the *SEMA6B* gene reversed these negative processes. Li et al. discovered that high *SEMA6B* expression was associated with unfavorable prognosis for patients and adverse polarization of the tumor immunosuppressive microenvironment in colorectal cancer (CRC) patients [39]. These observations suggest that the abatement of SEMA6B expression may be considered an option for cancer treatment; nevertheless, it needs to be further investigated.

Interestingly, plexin-A4, which confers signaling from SEMA6A and SEMA6B, seems to form stable complexes with FGFR1 and VEGFR2 receptors and thus transactivates their signaling pathways [40]. As a result of the intrinsic activity of the SEMA6B–plexin-A4–FGFR1/VEGFR2 axis, endothelial cells (HUVECs) and U87MG glioblastoma cells acquired pro-proliferative stimuli that assembled for pro-angiogenic and pro-tumorigenic signaling in the tumor microenvironment. Indeed, the silencing of plexin-A4 in human umbilical vein endothelial cells resulted in a reduction in proliferation and angiogenesis [40]. Similarly, silencing SEMA6B/plexin-A4 in U87MG glioblastoma cells diminished tumor-forming abilities. In other words, due to reciprocal plexin-A4 coreceptor activity, SEMA6B plays a substantial role in cancer vascularization and responsiveness to environmental stimuli. Based on these

observations, the authors of the study suggested that the plexin-A4-SEMA6 axis may be exploited as a target for antitumor therapies [40].

An attractive approach to regulating human *SEMA6B* gene expression was proposed in papers by Collet et al. [41] and Murad et al. [42][43]. These studies investigated the use of specific ligands (agonists) that bind to peroxisome proliferator-activated receptors (PPARs) and 9-cis retinoic acid receptor (RXR). Experiments with the PPARα agonist clofibrate and human glioblastoma T98G cells have shown a strong downregulation of *SEMA6B* gene expression [41]. In follow-up experiments, similar effects were observed in human MCF-7 breast adenocarcinoma cells (the highest expression of *SEMA6B* among cancer cell lines) treated with either fenofibrate (a PPARα activator) or troglitazone (a PPARγ ligand) [42]. Combined treatment of MCF-7 cells with fenofibrate and retinoic acid (RXR agonist) for 72 h substantially decreased SEMA6B protein expression by almost 40%, while in cells exposed to troglitazone and retinoic acid, it decreased SEMA6B protein expression by 70%. The efficacy of this strategy was confirmed in a study on animals aimed at downregulating SEMA6B in rat skeletal muscles [43]. The authors demonstrated the binding of PPARα to the putative PPAR response element (PPRE) in the *SEMA6B* promoter of rat skeletal muscle after fenofibrate treatment. Furthermore, these effects translated into a 2.5-fold lower expression of SEMA6B in the muscles.

3. Semaphorin 6C

Semaphorin 6C is the least well-known member of the SEMA6 family. It triggers cellular signaling through plexin-A1. Predominantly, widespread expression of SEMA6C has been found in the murine brain, developing embryos, and adult animals, suggesting its important role in neurogenesis and synapse stabilization or formation at postnatal stages [44][45]. Indeed, early functional studies in 2002 focused on the identification of a new axon's guiding cues delivered new classes of SEMAs, namely SEMA6C and SEMA6D [45]. Substantially, the function of SEMA6C was associated with the inhibition of axonal extension of PC12 cell-differentiated neurons and their outgrowth via directed growth cone collapse in cultured rat hippocampal neurons and rat cortical neurons in a dose-dependent manner. Complementary studies by Svensson et al. [46] on the role of SEMA6C in the peripheral nervous system unveiled the presence of strong immunoreactivity against SEMA6C at the neuromuscular junction of rat anterior tibial and hemidiaphragm muscles. Furthermore, following muscle denervation, the expression of SEMA6C decreased, indicating neuronal downstream regulation of SEMA6C in synapses of motoneurons. This evidence strongly supports the idea of the importance of SEMA6C in neuromuscular communication [46].

4. Semaphorin 6D in the Immunological Landscape of Tumors

SEMAs and plexin-A1/A4, pairs of cellular ligand–receptor molecules, are noticeably represented on cells of the innate and adaptive immune systems, such as granulocytes, macrophages, dendritic cells (DCs), and $CD4^+$ and $CD8^+$ lymphocytes [47][48][49]. Their multivariate combination of interactions mediates multiple cell–cell contacts among immune and cancer cells and may confer variable clinical therapy responses within the TME. Indeed, for

example, the expression of SEMA4A in the cancer niche of NSCLC or the use of recombinant rSEMA4A improves the response to anti-PD-1 monotherapy and boosts the effector function of tumor-infiltrating $CD8^+$ T cells in vitro [49]. On the other hand, SEMA3A secreted by a variety of human tumor cells (lung, breast, prostate, glioblastoma, and colorectal cancer cells) has been shown to inhibit tumor-$CD4^+$/$CD8^+$ lymphocyte interactions and T-cell activation and proliferation [47].

The preliminary role of plexin-A1 in immune cells was reported by the Ting group in 2003 [50]. In this research, authors emphasized the significant and exclusive presence of plexin-A1 in DCs over other immune cells analyzed, indicating plexin-A1 as a marker to distinguish DCs from other APCs. Moreover, the authors identified major histocompatibility complex (MHC) class II transactivator (CIITA), regarded as the master coactivator of MHC class II genes at the promoter level, as the activator of the *Plxna1* promoter (8-10-fold activation) in murine cells. To further assess the role of plexin-A1 in immune function, the authors employed the interference RNA technique to downregulate the cellular level of plexin-A1. The resulting experimental data demonstrated plexin-A1 as a necessary molecule for optimal priming of T-cell activation by protein- or peptide–antigen-pulsed DCs (**Figure 2**A).

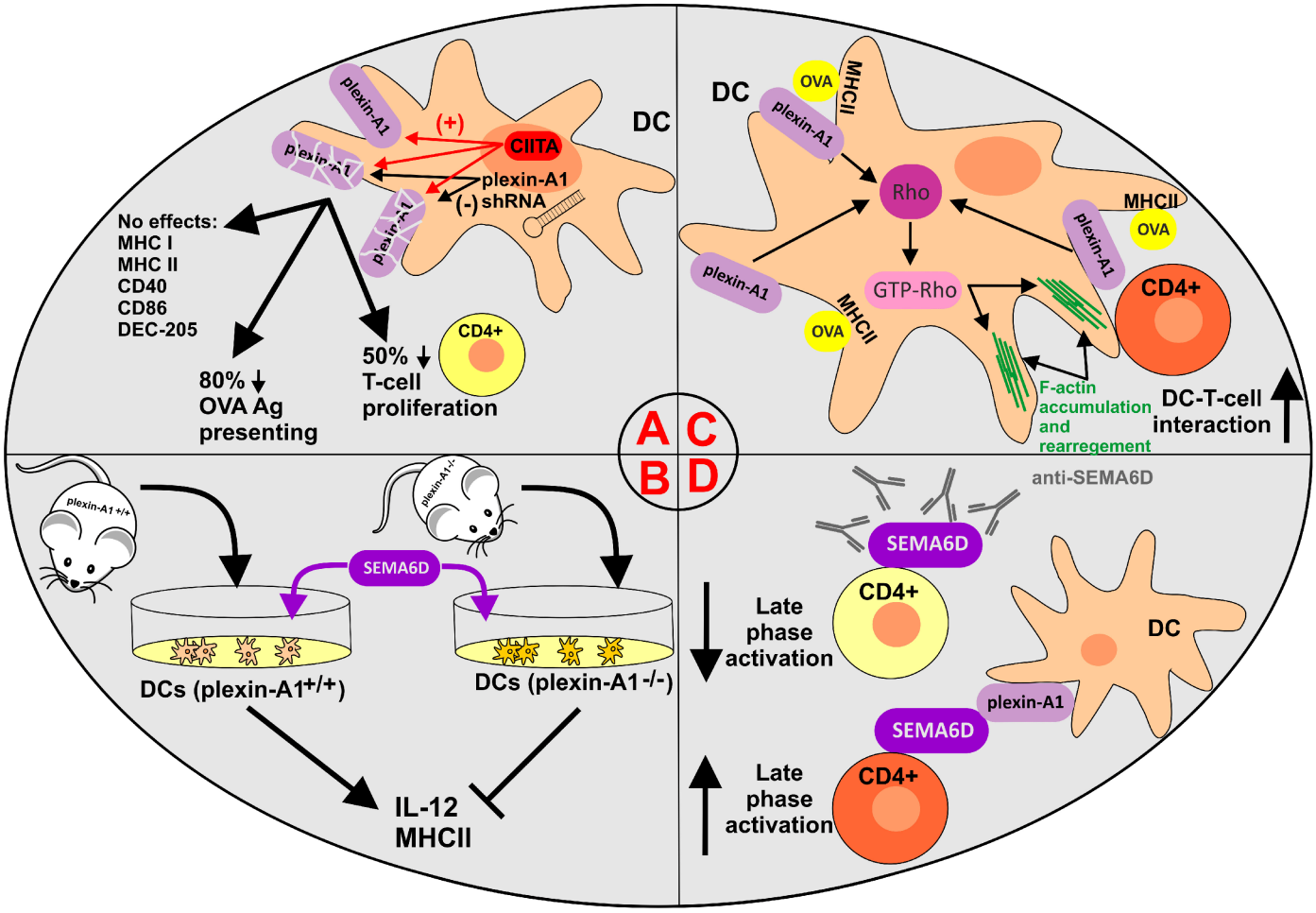

Figure 2. Role of plexin-A1 and SEMA6D components in various immunological processes involving dendritic cells and T cells. (**A**) CIITA activates the plexin-A1 promoter in murine DCs. Stably transfected DCs with plexin-A1 shRNA exhibit compromised OVA antigen presentation and T-cell activation. (**B**) Plexin-A1-deficient DCs isolated

from plexin-A1$^{-/-}$ mice exhibit compromised IL-12 production and MHC II expression. (**C**) Schematic representation of the role of plexin-A1 in controlling the priming of DC–T-cell interactions. Plexin-A1 is dispensable for Rho and F-actin accumulation and polarization in the immunological synapse of DCs preceding dendrite formation and T-cells adhesion. (**D**) Targeting of Sema6D–plexin-A1 pairing in cocultured OVA-triggered DCs and $CD4^+$ cells by anti-SEMA6D antibodies reduced the proliferation rate of T-cells and diminished their activation rate in the late phase of activation (4–6 days). (+)—stimulation; (-) or ⊤—inhibition; ↑—upregulation; ↓—downregulation.

The natural counterparts for SEMA6D-positive $CD4^+$ cells are plexin-A1-bearing DCs, which also coexpress plexin-A4, SEMA3A and Np1 receptors. Thus, such reciprocal T-cell–DC cell–cell interactions via Sema6D–plexin-A1 contacts are expected. According to Takegahara and colleagues [51], the incubation of dendritic cells with recombinant soluble SEMA6D induced IL-12 production and upregulation of MHC class II expression. Complementary experiments using plexin-A1$^{-/-}$ deficient dendritic cells in the presence of SEMA6D significantly restricted SEMA6D binding to DCs and reduced IL-12 production and MHC class II upregulation. Moreover, cytokine production (IL-2, IL-4, IFNγ) and proliferation by $CD4^+$ cells prepared from draining murine lymph nodes were considerably reduced as a result of compromised sensing of environmental stimuli by dendritic cells derived from plexin-A1$^{-/-}$ mice (**Figure 2**B).

Recent evidence on the role of plexin-A1 in DC-mediated T-cell activation addressed the possible molecular mechanism involving the activation of Rho GTPases (known to regulate the actin cytoskeleton) [52]. A study by Eun et al. documented that plexin-A1 localizes to the DC membrane and focal adhesions during DC-T-cell contacts following antigen pulse. Furthermore, coupled DC-T-cell conjugates demonstrated F-actin accumulation at the DC-T-cell interface in DCs but not plexin-A1 shRNA DCs. Following antigen pulsing (ovoalbumin, OVA), DC–T-cell pairs triggered an efficient activation of Rho in DCs. Complementary experiments using plexin-A1-deprived DCs displayed over 50% lower F-actin polarization in DCs, which is vital for the creation of immunological synapses during T-cell–DC interactions [53]. Additionally, OVA-pulsed plexin-A1 shRNA-treated DCs exhibited a lower activation of Rho (interestingly, not of Rac or Cdc42) than control shRNA-treated DCs. Finally, pretreatment of DCs with the Rho inhibitor C3 greatly reduced the accumulation of F-actin in DCs at the interface with T-cells. Collectively, the present study underlines the regulatory role of the plexin-A1–Rho axis in cytoskeletal rearrangements and highlights the major role of this mechanism during the creation of immunological synapses and DC–T-cell interactions (**Figure 2**C).

Interestingly, semaphorin 6D (possibly paired with plexin-A1) has also been attributed to the generation of immunological memory via the activation of the late phases of the T-cell immune response [54][55]. According to O'Connor et al., the activation of $CD4^+$ T cells enhanced SEMA6D expression in vivo and vice versa [54]. As expected, targeting SEMA6D with specific anti-SEMA6D antibodies diminished endogenous T-cell signaling mediated by phosphorylation of the linker of activated T-cells (pLAT; possibly also c-Abl) and accounted for the reduction in the late phase of T-cell activation (**Figure 2**D).

Collectively, the presented experimental results describe plexin-A1 as a functional receptor for SEMA6D in the immune landscape and suggest the participation of the SEMA6D–plexin-A1 pairing in the early phases of immune

reactions via T-cell–DC contacts. Importantly, activated $CD4^+$ T-cells can increase antitumor immunity by promoting pro-inflammatory cross-presenting dendritic cells (DCs) and thus strengthen the antitumor effector functions of $CD8^+$ [56].

Apart from plexin-A1–SEMA6D interactions, plexin-A4 may also act as a receptor for SEMA6D, as both molecules exhibit widespread expression on the surface of interacting immune cells, including T cells, dendritic cells (DCs), and macrophages [57]. Kang et al. investigated the role of SEMA6D and plexin-A4 in the polarization of macrophages toward the anti-inflammatory subtype [58]. Notably, these regulatory mechanisms employ SEMA6D to function as a receptor itself via reverse signaling [59]. Macrophages can be classified into two distinct subtypes, pro-inflammatory and anti-inflammatory, distinguished by their secretion of various cytokines and mediators and by different factors that drive their polarization into the respective subtype [60]. The process of macrophage polarization into the appropriate subtype is also subject to regulation by alterations in specific metabolic pathways. For instance, in the case of anti-inflammatory macrophages, IL4-induced upregulation of PPARγ and activation of mTOR kinase led to modifications in fatty acid metabolism, which are vital for the polarization process [61][62]. Kang et al. revealed that the expression of SEMA6D affects the direction of macrophage polarization, and its absence disrupts the immune response. They emphasized the significance of the mTOR–SEMA6D–PPARγ axis in linking immunity and metabolism during macrophage polarization (see **Figure 3**). This axis is under the regulation of mTOR kinase, which orchestrates the expression of SEMA6D. In turn, SEMA6D expression affects PPARγ expression and metabolic processes associated with fatty acid uptake and lipid metabolism remodeling. The lack of SEMA6D leads to impaired polarization of macrophages toward an anti-inflammatory state and heightened pro-inflammatory polarization. In this context, SEMA6D functions as a receptor in reverse signaling through its cytoplasmic domain and the c-Abl kinase to regulate PPARγ expression. The interaction between SEMA6D and its ligand plexin-A4 stimulates reverse signaling, thereby contributing to the polarization of macrophages into the anti-inflammatory subtype [58]. The process of polarizing anti-inflammatory macrophages, in which SEMA6D plays a crucial role, as demonstrated both in vitro and in vivo, holds potential implications for understanding the function of tumor-associated macrophages (TAMs). Manipulating the mTOR–SEMA6D–PPARγ axis, which connects macrophage immunological function and metabolism, offers promise in modulating immune responses and therapeutically influencing macrophage polarization not only in cancer but also in various other diseases. However, the precise role of SEMA6D in macrophage polarization within the tumor microenvironment remains incompletely elucidated.

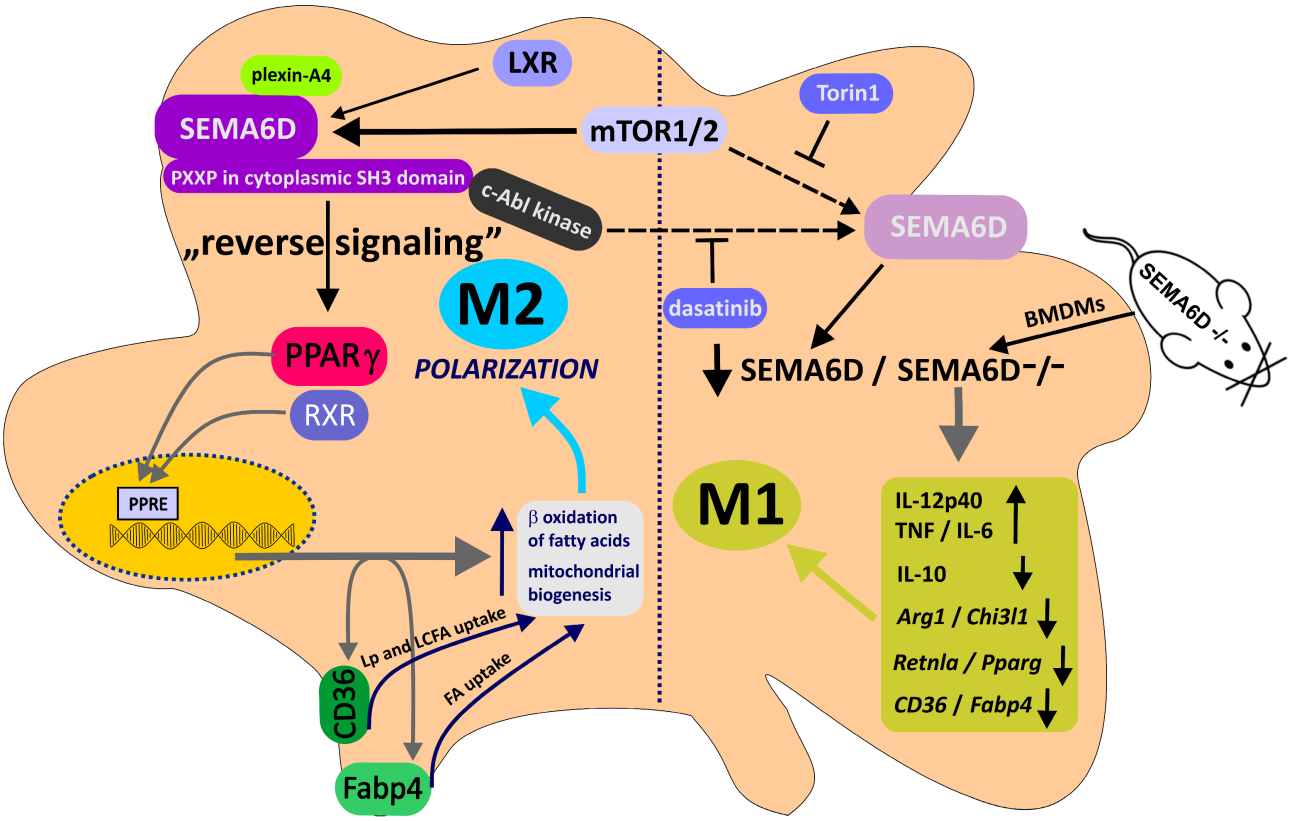

Figure 3. Illustration of the mTOR–Sema6D–PPARγ signaling pathway in controlling macrophage polarization toward anti-inflammatory (antitumorigenic) phenotype M2 via regulation of fatty acid uptake and metabolic reprogramming. mTOR is a critical molecule that controls SEMAD6 expression and regulates the metabolic status of the cell to promote polarization of macrophages. Association of c-Abl tyrosine kinase with SEMA6D with the PXXP region of the cytoplasmic SH3 domain promotes reverse signaling, enhanced by binding plexin-A4. As a result of SEMA6D-driven reverse signaling, PPARγ expression increases and primes metabolic reprogramming events such as fatty acid biosynthesis and fatty acid uptake pathways (CD36, Fabp4). SEMA6D–PPARγ signaling is indispensable for metabolic reprogramming. Abnormalities in this pathway account for impaired fatty acid uptake and metabolic reprogramming, leading to aberrant macrophage polarization. Inhibition of upstream mTOR1/2 with Torin1 or activity of c-Abl tyrosine kinase with dasatinib suppresses the expression of PPARγ and the anti-inflammatory signature genes *Arg1* and *Chi3l1*. Similarly, mutation of the SH3 domain in SEMA6D or absence of SEMA6D accounts for defective anti-inflammatory macrophage polarization (low expression of anti-inflammatory signature genes: *Retnla*, *Il10*, and receptors *Cd36*, *Fabp4*) along with exaggerated inflammatory responses (IL-12p40, TNF, IL-6). mTOR1/2: mammalian target of rapamycin 1/2; PPARγ: peroxisome proliferator-activated receptor gamma; RXR: retinoid X receptor; PPRE: peroxisome proliferator hormone response elements; LXRα: liver X receptor alpha; Arg1: arginase 1; Chi3l1: chitinase 3 like-1; Retnla: resistin-like molecule alpha; CD36: receptor for lipoproteins; Fabp4: Lp: lipoproteins; FA: fatty acids; LCFA: long-chain fatty acids; BMDMs: bone marrow-derived macrophages. ⊤—inhibition; ↑—upregulation; ↓—downregulation.

Beyond the immune system, SEMA6D is involved in the development of the heart by promoting the proliferation of cardiomyocytes [63], preliminary stages of atrioventricular cushion mesenchyme development [64], and proper

myocardium organization by enhancing the migration of cardiac cells into trabeculae [59]. Conversely, the pathological proliferation of cells accompanying liver fibrosis has been associated with a higher level of SEMA6D in the blood of patients with chronic hepatitis C (CHC) [65]. Furthermore, SEMA protein levels (SEMA3C, SEMA5A, and SEMA6D) positively correlated with fibrosis stage in CHC. Indeed, antiviral therapy against HCV improved the stage of liver fibrosis and decreased serum SEMA3C/SEMA6D levels. Interestingly, the authors of the study concluded that SEMA6D may serve as a sensitive marker of liver injury in CHC (superior to APRI and FIB-4 in predicting the development of cirrhosis) [65].

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