



Review article

The SMYD family proteins in immunology: An update of their obvious and non-obvious relations with the immune system

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ABSTRACT

Epigenetics is an emerging field, due to its relevance in the regulation of a wide range of biological processes. The Su(Var)3–9, Enhancer-of-zeste and Trithorax (SET) and Myeloid, Nervy, and DEAF-1 (MYND) domain-containing (SMYD) proteins, named SMYD1, SMYD2, SMYD3, SMYD4 and SMYD5, are enzymes that catalyse methylation of histone and non-histone substrates, thereby playing a key role in gene expression regulation in many biological contexts, such as muscle development and physiology, haematopoiesis and many types of cancer. This review focuses on a relatively unexplored aspect of SMYD family members - their relation with immunology. Here, immunology is defined in the broadest sense of the word, including basic research on macrophage function or host immunity against pathogen infection, as well as clinical studies, most of which are centred on blood cancers.

1. Introduction

The Su(Var)3–9, Enhancer-of-zeste and Trithorax (SET) and Myeloid, Nervy, and DEAF-1 (MYND) domain-containing (SMYD) histone methyltransferases are a family of proteins that is composed by five members in mice and humans: SMYD1, SMYD2, SMYD3, SMYD4 and SMYD5. The SET domain has lysine-specific methyltransferase activity, while the MYND domain contains a zinc-finger motif able to bind proline-rich regions and mediate protein-protein interactions, as well as DNA binding. Thus, these enzymes act on histone and non-histone targets to regulate many biological processes, including muscle development and cancer (Spellmon et al., 2015). Indeed, SMYD2 (Yi et al., 2019) and SMYD3 (Bottino et al., 2020) are considered to have oncogenic properties in a myriad of cancer types, although they are dispensable for normal mouse development and adult life, probably due to its redundancy (Bagislar et al., 2016). In addition, SMYD proteins are very important for muscle physiopathology (Du et al., 2014). Here, we will focus on their function in immunology, in the broadest sense of the word, including basic mechanisms and clinical conditions related to the immune system.

2. Innate immune cells: macrophages

SMYD2, SMYD3 and SMYD5 seem to play a role in macrophages. Xu et al. identified SMYD2 as a negative regulator of macrophage activation

and M1 polarization. *Smyd2* upregulation abrogated macrophage production of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF α), among other proinflammatory cytokines, by catalysing H3K36 dimethylation on *Tnf* and *Il6* promoters and suppression of nuclear factor kappa B subunit 1 (NF κ B) and extracellular signal-regulated kinase (ERK) signalling (Xu et al., 2015). Li et al. studied macrophages in pathological conditions, such as exposure to the plastics industry widely-used chemicals bisphenol A and phthalate. These chemicals affect peritoneal macrophages by hampering their capacity to produce proinflammatory cytokines, express scavenger receptors and phagocytise. SMYD2 seemed to be involved in this process since SMYD2 knockdown or inhibition of its methyltransferase activity led to an expected decrease in H3K36 dimethylation and activation of IL-6 and TNF α expression, although the phagocytic capacity of the macrophages was unexpectedly reduced (Li et al., 2018).

In regard to SMYD3, Yıldırım-Buharalıoğlu et al. observed that 18 h after interferon gamma (IFN γ) treatment of colony-stimulating factor 1 (CSF1)-differentiated primary human macrophages, *SMYD3* mRNA levels decreased, in parallel with a reduction in cell proliferation (Yıldırım-Buharalıoğlu et al., 2017). Furthermore, under hyperglycaemic conditions, SMYD3, together with SET7/9, activated S100 calcium binding protein A12 (*S100A12*) expression by methylation of the *S100A12* promoter in classically activated M1 macrophages (Mossel et al., 2020).

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With respect to SMYD5, Stender et al. demonstrated that SMYD5 is associated with the nuclear receptor corepressor (NCoR) complex and recruited to Toll-like receptor (TLR)4-responsive genes in macrophages, where it actively participates in the basal repression of TLR4-responsive promoters by catalysing histone 4 lysine 20 trimethylation (H4K20me3). When TLR4 is activated, PHD finger protein 2 (PHF2) demethylates H4K20me3 to induce TLR4-dependent gene expression. According to their results, SMYD5 may be a negative regulator of macrophage inflammatory responses (Stender et al., 2012).

3. Host-pathogen interactions

In addition to macrophages, SMYD2 and SMYD3 are also implicated in host-pathogen interactions. Infection with *Leishmania donovani*, the intracellular parasite responsible for leishmaniasis, activated the expression of *Smyd2* via *c-Myc* in murine cell lines and primary macrophages. This parasitic infection also caused histone 3 lysine 36 (H3K36) dimethylation at the TNF α promoter, probably by the enzymatic action of SMYD2. Pharmacological inhibition of SMYD2 using AZ505 enhanced the protective inflammatory response in infected macrophage cell lines and decreased parasite multiplication in infected mice. Thus, SMYD2, along with other methyltransferases, aids *Leishmania donovani* in the process of infecting the host (Parmar et al., 2020). Moreover, using human CD4+ cells lines and T cells from human immunodeficiency virus type 1 (HIV-1)-infected donors, Boehm et al. demonstrated that SMYD2 mediates H4K20 monomethylation in the HIV-1 promoter located in the 5' long terminal repeat. H4K20 monomethylation promotes association of the reader protein L3MBTL histone methyl-lysine binding protein 1 (L3MBTL1) with the 5' long terminal repeat. The presence of SMYD2 is required for this association and necessary for HIV latency, since SYMD2 knockdown or inhibition reactivates the virus. Thus, SMYD2 positively regulates HIV-1 latency (Boehm et al., 2017).

SMYD3 plays a role in host immunity in relation to the human T-cell lymphotropic virus type 1 (HTLV-1, also known as human T-cell leukaemia type 1). This virus is linked to leukemogenesis, among other pathological processes. Yamamoto et al. revealed that there is endogenous SMYD3 expression in T cell lines and primary T cells, in which it directly interacts with HTLV-1 Tax and supports its cytoplasmic localisation. By controlling Tax subcellular localisation, SMYD3 permits or hampers its interaction with cytoplasmic or nuclear proteins (Yamamoto et al., 2011). Additionally, Nagata et al. claimed that SMYD3 is involved in the epigenetic regulation of inducible regulatory T (iTreg) cells. SMYD3 catalysed histone 3 lysine 4 (H3K4) trimethylation in the promoter region and conserved the noncoding DNA sequence of the *foxp3* gene and regulated its expression in a transforming growth factor-beta1/mothers against decapentaplegic homolog 3 (TGF β 1/Smad3)-dependent manner. Hence, inhibition of SMYD3 by siRNA impaired iTreg cell formation *in vitro*. Accordingly, SMYD3 KO mice infected with the respiratory syncytial virus displayed exaggerated inflammatory responses and aggravation of the disease, due to the key role of iTreg in this viral infection (Nagata et al., 2015).

4. Blood cell cancers

Regarding immune cell-related cancer, recurrent mutations in *SMYD1* were found in a group of patients with splenic marginal zone lymphoma, which is a type of B-cell non-Hodgkin lymphoma (Peveling-Oberhag et al., 2015). The mechanism by which this mutations affect SMYD1 function remains to be clarified and, therefore, it is unknown if SMYD1 behaves as an oncogene or as a tumour suppressor in lymphoma.

With reference to malignant haematopoiesis, Velinder et al. showed that SMYD2 methylates growth factor independent 1 transcription repressor (GFI1), which is a master regulator of normal and malignant haematopoiesis. The proposed mechanism was that this specific methylation in GFI1 protein provokes recruitment of lysine demethylase 1A (LSD1) and repression of the transcription of GFI1-targeted genes,

leading to normal and/or malignant haematopoiesis (Velinder et al., 2016).

High expression levels of SMYD2 were described in many types of leukaemia by Brown et al. In B-cell acute lymphoblastic leukaemia there was a positive correlation between high *SMYD2* and low overall patient survival. Haematopoietic stem cell-specific SMYD2 KO mice displayed aberrant haematopoiesis caused by apoptosis induction and changes in gene expression. Indeed, SMYD2 controls gene expression programmes related to wingless-related integration site (WNT) regulation of haematopoietic stem cells proliferation, in part by its association with Frizzled Receptor 2 (FZD2) (Brown et al., 2020). Another study confirmed the previous results (Brown et al., 2020); *SMYD2* was overexpressed in acute lymphoblastic leukaemia compared to non-malignant bone marrow and high *SMYD2* expression negatively correlated with overall survival in acute lymphoblastic leukaemia. A decrease in *SMYD2* expression was observed 29 days after chemotherapy in most of the patients who responded to the treatment (leukaemia remission was determined by the presence of less than 5% of leukaemic cells in bone marrow) (Sakamoto et al., 2014). Similar results were obtained by Zhang et al. who observed overexpression of *SMYD2* in the bone marrow of children with B lineage acute lymphoblastic leukaemia, which was associated with bad prognosis (including increased white blood cell count and less overall survival and tumour-free survival) and a reduction of SMYD2 expression levels after remission (Zhang et al., 2020). Animal studies demonstrated that, although germ-line SMYD2 KO mice are viable, healthy and display normal haematopoiesis, SMYD2 deficiency delayed and reduced penetrance of death due to MLL-AF9/NRas^{G12D}-induced acute myeloid leukaemia. After disease establishment, both wild-type (WT) and SMYD2 KO cells gave rise to indistinguishable acute myeloid leukaemia phenotypes, but SMYD2 deletion caused competitive disadvantages over their WT counterparts *in vitro* and *in vivo*. The authors also described direct binding of Myc to *Smyd2* promoter as a possible molecular mechanism for this phenotype (Bagislar et al., 2016). Moreover, Oliveira-Santos et al. found that both *SMYD2* and *SMYD3* were upregulated in malignant B cells from the same patients with chronic lymphocytic leukaemia, indicating that these two SMYD proteins may be controlled by the same molecular mechanism in this biological context. Interestingly, patients with very low expression of *SMYD2* and *SMYD3* displayed elevated white blood cell counts and a complex karyotype (Oliveira-Santos et al., 2016). Furthermore, Hay et al. noticed that premalignant thymus from RUNX family transcription factor 2 (RUNX2)/myelocytomatosis oncogene (MYC) transgenic mice overexpress *Smyd2* and, by extrapolating data

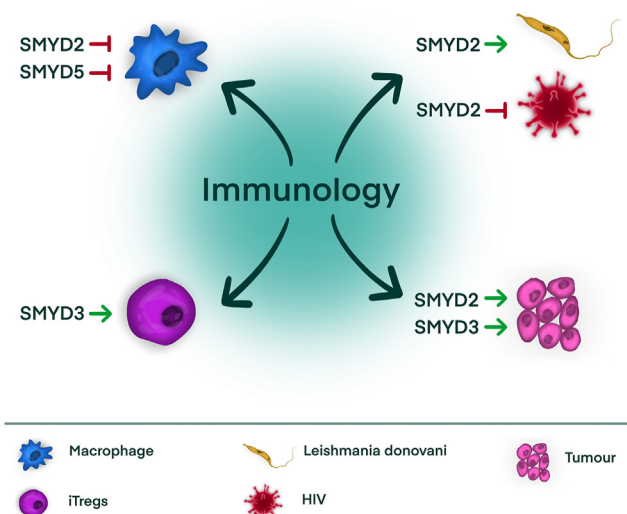


Figure 1. Schematic representation of the main findings regarding SMYD proteins in immunology. Abbreviations: iTreg; inducible regulatory T cells, HIV; human immunodeficiency virus.

Table 1. Studies on SMYD proteins and the main findings related to immunology.

SMYD	Model	Main findings	References
SMYD1	pGL-2 and pGL-IO cell lines	SMYD1 expression in cytotoxic T lymphocytes	(Hwang and Gottlieb 1997)
SMYD1	Muscle-specific <i>Smyd1</i> KO mice, N = 3	Downregulation of <i>Il-6</i> , <i>Tnfα</i> , <i>Ccl6</i> , <i>Ccl7</i> , <i>Ccl9</i> , <i>Foxp3</i> , <i>Bcl11A</i> , <i>RunX1</i> and <i>Cbfb</i> in brown adipose tissue	(Rasmussen and Tucker 2018)
SMYD1	Yak, N = 48	Copy number variations in <i>SMYD1</i>	(Wang et al., 2019)
SMYD1	AnWj- negative humans, N = 6	R320Q substitution in SMYD1	(Yahalom et al., 2018)
SMYD1	Patients with velocardiofacial syndrome, N = 88	Identification of <i>SMYD1</i> as a putative pathogenic gene	(Wu et al., 2019)
SMYD1	Patients with splenic marginal zone lymphoma, N = 26	Recurrent mutations in <i>SMYD1</i>	(Peveling-Oberhag et al., 2015)
SMYD2	Monkey Cos7L cell line	SMYD2 methylation of GF11 provokes recruitment of LSD1 and repression of the transcription of GF11 target genes, leading to normal and/or malignant haematopoiesis	(Velinder et al., 2016)
SMYD2	C57BL/6 mice, N = 3	Upregulation of <i>Smyd2</i> abrogates macrophage production of IL-6 and TNFα by catalysing H3K36 dimethylation of Tnf and Il6 promoters and suppression of NFκB and ERK signalling	(Xu et al., 2015)
SMYD2	CD-1 female mice, N = 6-10	SMYD2 catalyses H3K36 dimethylation and inhibits IL-6 and TNFα expression after bisphenol A and phthalate exposure of peritoneal macrophages	(Li et al., 2018)
SMYD2	Human monocyte THP-1 cell line and human primary monocytes	The TNFα inhibitor etanercept acts by suppressing LPS-induced <i>CCL2</i> expression by decreasing protein levels of SMYD2 and H3K36me3 in the <i>CCL2</i> promoter region	(Lin et al., 2017)
SMYD2	Patients with leukaemia, N = 50 Haematopoietic stem cell-specific SMYD2 KO mice, N = 3-8	<i>SMYD2</i> overexpression in many types of leukaemia Positive correlation between high <i>SMYD2</i> and low overall patient survival in B-cell acute lymphoblastic leukaemia Aberrant haematopoiesis in haematopoietic stem cell-specific SMYD2 KO mice	(Brown et al., 2020)
SMYD2	Patients with leukaemia, N = 83	<i>SMYD2</i> overexpression in acute lymphoblastic leukaemia Positive correlation between high <i>SMYD2</i> and low overall patient survival in acute lymphoblastic leukaemia Decrease in <i>SMYD2</i> expression 29 days after chemotherapy related to treatment response	(Sakamoto et al., 2014)
SMYD2	Patients with leukaemia, N = 134	<i>SMYD2</i> overexpression in B lineage acute lymphoblastic leukaemia, associated with bad prognosis Reduction of SMYD2 expression levels after remission	(Zhang et al., 2020)
SMYD2	SMYD2 KO mice, N = 12	Normal haematopoiesis in SMYD2 KO mice Reduced penetrance of death due to MLL-AF9/NRas ^{G12D} -induced acute myeloid leukaemia in SMYD2 KO mice Competitive disadvantages of SMYD2 KO acute myeloid leukaemia cells	(Bagislar et al., 2016)
SMYD2	Patients with leukaemia, N = 59	<i>SMYD2</i> and <i>SMYD3</i> upregulation chronic lymphocytic leukaemia Residual expression of <i>SMYD2</i> and <i>SMYD3</i> correlated with elevated white blood cells count and a complex karyotype	(Oliveira-Santos et al., 2016).
SMYD2	RUNX2/MYC transgenic mice, N = 3	<i>Smyd2</i> overexpression in premalignant thymus from RUNX2/MYC transgenic mice	(Hay et al., 2019)
SMYD2	Patients with leukaemia and hematopoietic TEX cell line	Low expression of SMYD2 in acute myeloid leukaemia negatively correlates with treatment response and probability of tumour-free survival SMYD2 KO human acute myeloid leukaemia cells are more resistant to DNA damage caused by irradiation due to transient quiescence	(Zipin-Roitman et al., 2017)
SMYD2	Female BALB/c mice, N = 3 Murine J774 macrophage cell line	<i>Smyd2</i> upregulation and H3K36 dimethylation at TNFα promoter after infection with <i>Leishmania donovani</i> SMYD2 inhibition protects against <i>Leishmania donovani</i> infection	(Parmar et al. 2020)
SMYD2	CD4+ T cells from patients infected HIV-1, HEK293T and J-Lat cell lines	SMYD2 mediates H4K20 monomethylation in the HIV-1 promoter located in the 5' long terminal repeat. H4K20me1 promotes association of L3MBTL1 with the 5' long terminal repeat SYMD2 inhibition reactivated HIV-1	(Boehm et al., 2017)
SMYD3	Human primary monocytes	Decreased <i>SMYD3</i> mRNA and cell proliferation 18 h after IFNγ treatment of CSF1 differentiated primary human macrophages	(Yildirim-Buharalioglu et al., 2017)
SMYD3	Patients with diabetes (T1DM and T2DM), N = 19-21	Under hyperglycaemic conditions, SMYD3 activates <i>S100A12</i> expression by methylation of <i>S100A12</i> promoter in M1 macrophages	(Mossel et al., 2020)
SMYD3	Many cell lines	SMYD3 in T cell lines and primary T cells directly interacts with HTLV-1 Tax and supports Tax cytoplasmic localisation	(Yamamoto et al., 2011)
SMYD3	Female C57BL/6 mice, N = 3	SMYD3 catalyses H3K4me3 in the promoter region of the <i>foxp3</i> gene and regulates its expression in iTreg cells Inhibition of SMYD3 impairs iTreg cell formation. SMYD3 KO mice infected with respiratory syncytial virus display	(Nagata et al., 2015)

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Table 1 (continued)

SMYD	Model	Main findings	References
		exaggerated inflammatory responses and aggravation of the disease	
SMYD3	Human Hodgkin lymphoma L1236 and L428 cell lines	SMYD3 participates in Hodgkin lymphoma development via H3K4 methylation at the promoter of 15-LOX-1	(Liu et al., 2012)
SMYD3	Human chronic lymphocytic leukaemia MEC1 cell line Patients with leukaemia, N = 20 Many human chronic lymphocytic leukaemia cell lines	SMYD3 upregulation by STAT3 in chronic lymphocytic leukaemia The STAT3-SMYD3 axis promotes carcinogenesis, since high SMYD3 levels showed a negative correlation with suppression of cell proliferation and invasion capacity Downregulation of the phosphorylation of STAT3 suppresses STAT3 binding to the SMYD3 promoter and SMYD3 expression	(Ma et al., 2019; Lin et al., 2019)
SMYD3	Human leukaemia K562 and HL- 60 cell lines	Overexpression of SMYD3 in a leukaemia cell line induces c-Met expression	(Zou et al., 2009)
SMYD3	Patients with human papilloma virus-negative squamous cell carcinoma of the head and neck, N = 364, and derived cell lines	Inhibition of SMYD3 causes CXCL9, CXCL10, CXCL11 and TAP1 upregulation in human papilloma virus -negative squamous cell carcinoma of the head and neck cell lines	(Vougiouklakis et al., 2017)
SMYD3	Human multiple myeloma U266 cell line	Triptolide downregulates SMYD3 in a human multiple myeloma cell line	(Zhao et al., 2010)
SMYD3	Human multiple myeloma U266 cell line	Berberine downregulates SMYD3 in a human multiple myeloma cell line	(Wang et al., 2016)
SMYD4	Patients with rheumatoid arthritis	SMYD4 overexpression in TNF α -stimulated synovial fibroblasts from patients with rheumatoid arthritis	(Araki et al., 2018)
SMYD5	Primary murine macrophages and many cell lines	SMYD5 basal repression of TLR4-responsive promoters in macrophages by catalysing H4K20me3	(Stender et al., 2012)

N indicates the number of subjects per experimental group. Abbreviations: KO; knockout, IL-6; interleukin-6, TNF α ; tumour necrosis factor- α , Ccl6; chemokine (C-C motif) ligand 6, Ccl7; chemokine (C-C motif) ligand 7, Ccl9; chemokine (C-C motif) ligand 9, Foxp3; forkhead box P3, Bcl11A; BAF chromatin remodelling complex subunit BCL11A, RunX1; RUNX family transcription factor 1, Cbfb; core binding factor β , GFI1; growth factor independent 1 transcription repressor, LSD1; lysine demethylase 1A, H3K36me2; histone 3 lysine 36 dimethylation, NF κ B; nuclear factor kappa B subunit 1, ERK; extracellular signal-regulated kinase, LPS; lipopolysaccharide, CCL2; C-C motif chemokine ligand 2, H3K36me3; histone 3 lysine 36 trimethylation, RunX2; RUNX family transcription factor 2, Myc; myelocytomatosis oncogene, HIV-1; immunodeficiency virus type 1, H4K20me1; histone 4 lysine 20 monomethylation, L3MBTL1; L3MBTL histone methyl-lysine binding protein 1, IFN γ ; interferon gamma, CSF1; colony-stimulating factor 1, T1DM; type 1 diabetes, T2DM; type 2 diabetes, S100A12; S100 calcium binding protein A12, HTLV-1; human T-cell lymphotropic virus type 1/human T-cell leukaemia type 1, H3K4me3; histone 3 lysine 4 trimethylation, iTreg; inducible regulatory T cells, H3K4me; histone 3 lysine 4 methylation, 15-LOX-1; 15-Lipoxygenase-1, STAT3; signal transducer and activator of transcription 3, Cxcl9; C-X-C motif chemokine ligand 9, Cxcl10; C-X-C motif chemokine ligand 10, Cxcl11; C-X-C motif chemokine ligand 11, Tap1; transporter 1, ATP binding cassette subfamily B member, TLR4; Toll-like receptor 4, H4K20me3; histone 4 lysine 20 trimethylation.

from murine primary fibroblasts, they speculated that SMYD2 (but not SMYD5) may inhibit the senescence-like growth arrest caused by RUNX upregulation in RUNX2/MYC lymphoma, thereby acting as an oncogene (Hay et al., 2019). Indeed, altogether, these data indicate that SMYD2 has an oncogenic role in leukaemia.

On the contrary, Zipin-Roitman et al. observed that low expression of SMYD2 in acute myeloid leukaemia patients negatively correlated with treatment response and the probability of tumour-free survival. In addition, human acute myeloid leukaemia cells devoid of SMYD2 were more resistant to DNA damage caused by irradiation compared to control cells, and the mechanism leading to this resistance seemed to be the induction of transient quiescence. The decrease in SMYD2 levels led to a compensatory increase of SET domain-containing protein (SET)7/9 methyltransferase levels, suggesting an interplay between the two enzymes that induces quiescence of leukaemia cells, which then become more resistant to chemotherapy (DNA damage) (Zipin-Roitman et al., 2017).

SMYD3 plays a role in all three principal types of blood cancers: leukaemia, lymphoma and myeloma. Liu et al. described a mechanism by which SMYD3 participates in the development of Hodgkin lymphoma via H3K4 methylation at the promoter of 15-Lipoxygenase-1 (15-LOX-1). Deregulation of this protein is observed in many cancer and pathological immunological conditions, including lymphoma (Liu et al., 2012). SMYD3 is upregulated by signal transducer and activator of transcription 3 (STAT3) in chronic lymphocytic leukaemia. The STAT3-SMYD3 axis promotes carcinogenesis, since high SMYD3 levels show a negative correlation with suppression of cell proliferation and invasion capacity. Downregulation of the phosphorylation of STAT3 by means of the STAT3 inhibitor WP1066 suppressed STAT3 binding to the SMYD3 promoter,

consequently inhibiting SMYD3 gene expression (Ma et al., 2019; Lin et al., 2019). Ectopic overexpression of SMYD3 in a human leukaemia cell line induced mRNA expression of the c-Met oncogene, thereby providing a rationale to test SMYD3 inhibitors for the treatment of leukaemia (Zou et al., 2009).

Moreover, knockdown of SMYD3 caused mRNA and protein levels upregulation of C-X-C motif chemokine ligand 9 (CXCL9), C-X-C motif chemokine ligand 10 (CXCL10), C-X-C motif chemokine ligand 11 (CXCL11) and transporter 1, ATP binding cassette subfamily B member (TAP1) in human papilloma virus-negative squamous cell carcinoma of the head and neck cell lines, suggesting a role of SMYD3 in cytokine release (Vougiouklakis et al., 2017).

Natural compounds can affect SMYD3 expression in cancer. Zhao et al. attempted to elucidate the anti-tumoural mechanism of action of triptolide (the main active component of extracts from the Chinese herb *Tripterygium wilfordii* Hook.F) in a human multiple myeloma cell line and found that triptolide downregulates SMYD3 (Zhao et al., 2010). Furthermore, the isoquinoline plant alkaloid berberine also downregulated SMYD3 gene expression in the same cell line (Wang et al., 2016).

5. Immune responses in rheumatoid arthritis

Immune cells are directly involved in inflammatory disorders such as rheumatoid arthritis. The TNF α inhibitor etanercept (commonly used for the treatment of rheumatoid arthritis) diminished the protein levels of SMYD2 and also H3K36 trimethylation in the C-C motif chemokine ligand 2 (CCL2) promoter region in a lipopolysaccharide (LPS)-stimulated human monocytic cell line. These and other data presented by the

authors indicate that the mechanism of action of this drug involves suppression of LPS-induced *CCL2* expression by inhibiting SMYD2 and other methyltransferases (Lin et al., 2017).

Nothing is known about the function of SMYD4 in relation to the immune system, either in health or in disease. There is only one article that mentions SMYD4 as a methyltransferase upregulated in TNF α -stimulated synovial fibroblasts from patients with rheumatoid arthritis compared to equally stimulated synovial fibroblasts from patients with osteoarthritis. No further insights about the biological meaning of this alteration in SMYD4 expression are offered by the authors (Araki et al., 2018).

6. SMYD1 and immune responses in other biological contexts

Finally, not-so-obvious relations of *Smyd1* with the immune system have been described. Due to its key role in cardiac and skeletal muscle development and regeneration (Du et al., 2014), muscle-specific *Smyd1* KO mice exhibited multiple defects of skeletal muscle, that finally led to perinatal death. Interestingly, trunk muscles were substituted with brown adipose tissue (BAT), and downregulation of key adaptive and innate immune transcription factors for BAT development and functionality, such as *Il-6*, *TNF α* , chemokine (C–C motif) ligand 6 (*Ccl6*), chemokine (C–C motif) ligand 7 (*Ccl7*), chemokine (C–C motif) ligand 9 (*Ccl9*), forkhead box P3 (*Foxp3*), BAF chromatin remodelling complex subunit BCL11A (*Bcl11A*), RUNX family transcription factor 1 (*Runx1*) and core binding factor β (*Cbfb*), was observed (Rasmussen and Tucker 2018). Furthermore, whole-genome resequencing of yak populations (*Bos grunniens*, ruminant animals that are evolutionarily adapted to high altitudes and, therefore, hypoxia) identified copy number variations in *SMYD1*, which may be involved in immune responses, as well as hypoxia adaptation (Wang et al., 2019). A cluster of patients with AnWj-negative blood group antigen, which is associated with lymphoid malignancies, immunologic disorders and autoimmune haemolytic anaemia, presented a R320Q substitution in *SMYD1*. This gene variant seems to be the base of an inherited form of the AnWJ-negative blood group phenotype, although the molecular mechanism is unknown (Yahalom et al., 2018). It has also been suggested that *SMYD1* is involved in the pathogenesis of velocardiofacial syndrome, a disease with heterogeneous genotypes and phenotypes, which could include immune deficiency (Wu et al., 2019).

7. Conclusions and further directions

Most of the data about SMYD protein function in immunology are related to blood cancers, since the involvement of these methyltransferases in cancer is unquestionable. Nevertheless, basic research regarding their function in immune cells is scarce, with few publications studying macrophages and T cells.

Since few studies explored the role of SMYD4 and SMYD5 in immunology, here we have mainly focused on SMYD1, SMYD2 and SMYD3. The murine *Smyd1* gene, also known as *Bop*, was described in the '90s (Hwang and Gottlieb 1995). The *Smyd1* gene was linked to T lymphocytes, and the authors demonstrated that this gene produces a presumably non-coding cDNA and two protein-coding variants; one protein variant expressed in cytotoxic T lymphocytes that derives from alternative splicing, as well as a second protein variant expressed in cardiac and skeletal muscle, the transcription of which is activated by a different promoter (Hwang and Gottlieb 1997). Although the role of the cytotoxic T lymphocytes variant has not been further explored, mutations on *SMYD1* have been linked to a wide range of diseases where the immune system plays a key function, suggesting that SMYD1 coordinates immune responses by mechanisms yet to be discovered.

Most of the data concerning the relationship between SMYD2 and the immune system refer to its methyltransferase activity on H3K36, which was initially described to restrain cell proliferation (Brown et al., 2006). Nonetheless, SMYD2 enzymatic activity is not restricted to H3K36, and this enzyme can also act on histone 4 lysine 20 (H4K20), among other

targets (Boehm et al., 2017; Yi et al., 2019). Through its methyltransferase activity, SMYD2 helps *Leishmania donovani* to infect the host organism (Parmar et al., 2020) and activates HIV-1 latency (Boehm et al., 2017). Furthermore, the majority of the data indicates that SMYD2 acts as an oncogene in blood malignancies, although there is some controversy. Despite that, selective SMYD2 inhibitors, such as compound LLY-507 (Nguyen et al., 2015), which have been successfully used to inhibit cell growth in other cancer types (Bayo et al., 2019; Kojima et al., 2020), could be tested in cell cultures and animals models of blood malignancies.

In addition, SMYD3 activity is linked to the immune system in many ways, including macrophage function, T cells and blood cancers. Its role as an oncogene seems to be relevant to immune system-related malignancies and the use of selective SMYD3 inhibitors will confirm that it is a cancer driver. Furthermore, SMYD3 function in iTreg is worth studying, since it might provide insights into host-pathogen interactions, but also a better understanding of tumorigenesis, since iTregs are recruited by many tumours (Deng 2018).

In conclusion, all the data presented in this review (summarised in Figure 1 and Table 1) indicate that SMYD family members do have relevant functions in immunology, in health and disease, but they need to be further explored. Future research may also assess whether they can be targeted in pathologies in which the immune system is crucial.

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