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Unravelling the immunological roles of dipeptidyl peptidase 4 (DPP4) activity and/or structure homologue (DASH) proteins

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Summary

Dipeptidyl peptidase (DPP) 4 (CD26, DPP4) is a multi-functional protein involved in T cell activation by co-stimulation via its association with adenosine deaminase (ADA), caveolin-1, CARMA-1, CD45, mannose-6-phosphate/insulin growth factor-II receptor (M6P/IGFII-R) and C-X-C motif receptor 4 (CXC-R4). The proline-specific dipeptidyl peptidase also modulates the bioactivity of several chemokines. However, a number of enzymes displaying either DPP4-like activities or representing structural homologues have been discovered in the past two decades and are referred to as DPP4 activity and/or structure homologue (DASH) proteins. Apart from DPP4, DASH proteins include fibroblast activation protein alpha (FAP), DPP8, DPP9, DPP4-like protein 1 (DPL1, DPP6, DPPX L, DPPX S), DPP4-like protein 2 (DPL2, DPP10) from the DPP4-gene family S9b and structurally unrelated enzyme DPP2, displaying DPP4-like activity. In contrast, DPP6 and DPP10 lack enzymatic DPP4-like activity. These DASH proteins play important roles in the immune system involving quiescence (DPP2), proliferation (DPP8/DPP9), antigen-presenting (DPP9), co-stimulation (DPP4), T cell activation (DPP4), signal transduction (DPP4, DPP8 and DPP9), differentiation (DPP4, DPP8) and tissue remodelling (DPP4, FAP). Thus, they are involved in many pathophysiological processes and have therefore been proposed for potential biomarkers or even drug targets in various cancers (DPP4 and FAP) and inflammatory diseases (DPP4, DPP8/DPP9). However, they also pose the challenge of drug selectivity concerning other DASH members for better efficacy and/or avoidance of unwanted side effects. Therefore, this review unravels the complex roles of DASH proteins in immunology.

Keywords: antigen presentation/processing, CD26, co-stimulation, DPP4 activity and/or structure homologue proteins (DASH), signal transduction

The dipeptidyl peptidase (DPP)4 family of DPP4 activity and/or structure homologue (DASH) proteins

Within the last two decades a number of enzymes have been discovered to also display DPP4-like activity or are structural homologues to DPP4. These enzymes/proteins are referred to as DPP4 activity and/or structure homologue (DASH) proteins, and can be grouped into the *DPP4* gene family and non-related DPP4-like enzymes.

DPP4 gene family

DPP4 belongs to the serine peptidase clan SC, subfamily 9b. Peptidases of the SC clan have a unique catalytic triad in the order of Ser, Asp and His located in an α/β-hydrolase fold compared to the chymotrypsin catalytic triad of His, Asp and Ser. Currently, six members have been identified as belonging to the dipeptidyl peptidase subfamily 9b, including DPP4, fibroblast activation protein alpha (FAP) [1], DPP8 [2], DPP9 [3], DPP4-like protein 1 (DPL1, DPP6, DPPX L, DPPX S) [4] and DPP4-like protein 2 [5]. Except for DPL1 and DPL2, all members display DPP4-like activity with neutral to basic pH optima and similar inhibition profiles [6,7].

DPP4 (CD26). DPP4 (CD26) is the best-known DASH protein and has been described in detail elsewhere [7–9].

DPP4 is a multi-functional protein involved in T cell activation by co-stimulation via its association with adenosine deaminase (ADA), caveolin-1, CARMA-1, CD45, mannose−6-phosphate/insulin growth factor-II receptor (M6P/IGFII-R) and C-X-C motif receptor 4 (CXC-R4). The proline-specific DPP4 also modulates the bioactivity of several chemokines, as well as neuropeptides and peptide hormones such as neuropeptide Y (NPY), substance P (SP), glucagon-like peptide (GLP)−1, GIP and glucagon. Indeed, several DPP4 inhibitors (gliptins) are currently on the market as anti-diabetic drugs [8–10]. Thus it is involved in glucose homeostasis, food uptake, anxiety, stress, cardiovascular, nociception and chemotaxis. The enzyme comprises 766 amino acids and is a type II transmembrane glycoprotein that has also a soluble-shedded form in serum. It has a molecular weight of 110 kDa and is active as a homodimer. It is distributed ubiquitously, with the highest expression in kidney, lung, liver and small intestine, whereas low expression is found in brain, heart and skeletal muscle. According to kinetic analysis, DPP4 has the highest selectivity for NPY and PYY. The human gene location of DPP4 is 2q24.2 [8,11,12].

Fibroblast activation protein alpha (FAP). FAP, also referred to as seprase, has the highest sequence identity to DPP4 and is believed to arise from gene duplication due to its gene proximity being at 2q23 [6]. FAP is a transmembrane protein type II. It consists of 760 amino acids and forms a 170 kDa homodimer. Like DPP4, the monomeric, *N*-glycosylated 97 kDa subunits are proteolytically inactive, thus their proteolytic activities are dependent upon subunit association. Furthermore, FAP can form a heterodimeric membrane-bound proteinase complex with DPP4 [6,7,13]. FAP has been shown to readily hydrolyse NPY, BNP, substance P and PYY as well as to a lower-rate GLP-1 and GIP, whereas chemokines were not readily truncated or at a much slower rates by FAP [14]. In addition to DPP4 activity, FAP also exhibits gelatinase and collagenase activity, which is collagen type I specific. Furthermore, α-2-antiplasmin is a natural substrate of serum-FAP, cleaved at $\ldots Gly_{11}\text{-}Pro_{12}\text{-}\Downarrow\text{-}Asn_{13}\ldots$, confirming that its endopeptidase activity requires the sequence X_{aa}-Gly-Pro-$Y_{aa}\ldots$ [15–18]. The crystal structure of FAP has been elucidated, and comparison with the crystal structure of DPP4 points to a lower anchoring of substrates by Glu_{203}–Glu_{204} due to shielding effects of surrounding hydrophobic residues and lack of Asp_{663}. This, in turn, results in a lower exopeptidase activity and enables its endopeptidase activity as confirmed by site-directed mutagenesis with subsequent kinetic studies [19]. Although FAP expression is restricted to reactive stromal fibroblasts of epithelial cancers, subsets of bone and soft tissue sarcomas, activated stellate cells, arthritic chondrocytes, granulation tissue of healing wounds as well as de-differentiated adipocytes, co-expression of DPP4 and FAP in these cells results in the formation of a heteromeric complex [6,7,20,21]. The DPP4 activity is maintained by both enzymes in the complex as well as gelatine degradation by FAP [22]. In tumorigenic cells and wounds, this heteromeric complex is localised on the advancing portion of invadopodia that is believed to play an important role in tumour invasion, spreading of metastasis, angiogenesis and wound-healing, respectively [6,7,13,22]. Thus, to identify it as a pharmaceutical target, the expression of FAP has been investigated as potential biomarker in several types of cancers [6,7,13,23–25]. Interestingly, it was shown that stromal FAP is more prominent in early-stage colorectal cancer and smaller tumour xenografts, with increased expression of FAP being an adverse prognostic indicator in patients with advanced metastatic disease [26]. In contrast, prolonged survival of patients with breast cancer was associated with high FAP expression, whereas in cervical cancer FAP was correlated with increased dysplasia and carcinoma development, suggesting FAP being an invasion marker [7,13,26]. Co-localization of FAP and urokinase-type plasminogen activator receptor was detected in malignant melanoma by fluorescence resonance energy transfer (FRET), and the complex appeared to be dependent upon both cytoskeleton and integrins [27]. Knock-out mice of FAP confirmed its role in wound-healing; however, no change of phenotype was observed with regard to cancer [28]. Expression of FAP was found to be up-regulated by interleukin (IL)-1 and oncostatin M in arthritic chondrocytes from patients with osteoarthritis, while it was down-regulated in patients with systemic lupus erythematosus [21]. Recently, the role FAP in cartilage degradation could be elucidated in FAP-knock-out of tumour necrosis factor (TNF)-α transgenic mice [$FAP^{(-/-)}$ human TNF transgenic (hTNFtg) mice], as these animals revealed less cartilage degradation, but similar inflammation and bone erosion compared to wild-type hTNFtg mice [29]. Cleavage of α_2-anti-plasmin by soluble serum-FAP yields a more active form, thereby promoting fibrosis and scar formation [17,18]. Thus, FAP has an opposite physiological role compared to DPP4 that enhances fibrinolysis and scar resolution by activation of plasmin from plasminogen via a quintary complex of ADA, plasminogen 2, DPP4, urinary plasminogen activator (uPA/tPA) and plasminogen-receptor (Plg-R) [7]. In addition, FAP has been proposed to play a role in neutropenia and anaemia [30].

DPP8. DPP8 consists of 882 amino acids, and its homodimer has a molecular weight of 200 kDa [2,31]. So far, it has been suggested to be located in the cytoplasm as a soluble protein, and until now there has been no evidence for any secretion [2,6,7,13]. Recent proteomic screening has revealed phosphorylation of p-ephrin-B1 antibody (Tyr_{331}) and mitogen-activated protein kinase-activated protein kinase-2 (MAPK-APK-2) (Thr_{334}) [32]. Using several chromogenic substrates, DPP8 was shown to display DPP4-like activity similar to DPP4 [2,31]. Hydrolysis of NPY, GLP-1, GLP-2, peptide YY (PYY), interferon (IFN)-induced T cell alpha chemoattractant (ITAC), IFN-induced protein 10

(IP-10), stromal cell-derived factor (SDF)-1α and SDF-1β, but not of IFN-γ-induced monokine (MIG), growth-regulated protein b (Groβ) and eotaxin could be demonstrated *in vitro*, although the rate of cleavage was slower compared to DPP4, in particular for PYY [31,33,34]. Recent systematic degradomic analysis identified several *in-vivo* substrates involved in antigen presentation, signal transduction, cellular energy and nucleotide metabolism [34]. DPP8 mRNA is distributed ubiquitously, with its highest expression in testis, prostates, ovaries, placenta and brain [2,5,12,35]. Furthermore, it is up-regulated in activated lymphocytes [2]. However, its physiological function is currently unknown and still awaits further studies. The human gene localization is 15q22 [6].

DPP9. DPP9 has two variants comprised of 863 and 892 amino acids, respectively [6,36]. The longer DPP9 was found to be enzymatically active as a homodimer with an estimated molecular weight above 200 kDa. DPP9 lacks a transmembrane domain and is found intracellularly near the Golgi apparatus, although secretion from transfected cells has not yet been observed [3,6,12,36]. Recently, DPP9 was shown to be associated with mitochondria and to co-localize strongly with microtubules. Furthermore, DPP9 redistributed towards the ruffling plasma membrane upon stimulation with either phorbol 12-myristate 13-acetate or epidermal growth factor. DPP9 was also seen at the leading edge of migrating cells and co-localised with the focal adhesion proteins, integrin-1 and talin, resulting subsequently in phosphorylation of focal adhesion kinase and paxillin. This implicates DPP9 to be involved in tissue and tumour growth as well as metastasis [37]. A nuclear localization signal was identified at the extended *N*-terminal in an alternative spliced variant of long DPP9, targeting it to the nucleus [38]. Using several chromogenic substrates, DPP9 exhibited DPP4-like activity similar to DPP4, and was shown to truncate NPY, GLP-1, GLP2 and, to a far lesser extent, for PYY *in vitro* [6,12,31]. However, the cytoplasmic proteasome-derived antigenic peptide $RU1_{34–42}$, CXCL10, IL-1RA, S100-A10, SET nuclear proto-oncogene (SET) and human nucleobindin 1 (NUCB1) could be identified as natural substrates of DPP9, suggesting DPP9 to play an important role in peptide turnover and antigen presentation and inflammation [39,40]. Intriguingly, DPP9 was only able to hydrolyse the deglycosylated IL-1RA isoform. Furthermore, DPP9 was also shown to cleave enzymatically an as-yet unknown substrate involved in the phosphorylation of protein kinase B (Akt), thereby interfering with epidermal growth factor (EGF) signalling [41]. Its binding to Harvey rat sarcoma viral oncogene homologue (H-RAS) and small ubiquitin-like modifier (SUMO)1 also confirmed the involvement of DPP9 in signal transduction [42]. Recent systematic degradomic analysis and two-dimensional difference gel electrophoresis (2D DIGE) identified several substrates involved in antigen presentation, signal transduction, cellular energy and nucleotide metabolism [34,40]. Together with DPP4, and contrary to DPP8, DPP9 has a high specificity for the Val–Ala motif, as shown with substrate CSN8 [40]. DPP9 contains an Arg–Gly–Asp cell attachment motif and two potential glycosylation sites, although deglycosylation revealed no mass differences [3,5,36]. Like DPP4 and DPP8, DPP9 mRNA is distributed ubiquitously, with its highest expression in liver, heart and skeletal muscle and testis [3,6,35,36]. Its physiological function has not yet been elucidated, although an up-regulation of DPP9 mRNA was detected in human testicular tumour [35]. Gene knock-out in mice with inactive DPP9 turned out to be neonatal-lethal [43]. The gene is located on chromosome 19p13.3 [6,7,12,36].

So far, one cannot differentiate between DPP8 and DPP9 enzymatic activity due to the lack of selective inhibitors; however, DPP8/DPP9 activity could be detected in human leucocytes, rat brain, lung and testis, bovine testis, murine brain, organs of the immune system such as thymus, spleen, lymph nodes and peripheral blood mononuclear cells (PBMC), testis, skeletal and uterine muscles as well as colon [12,35,36,44–47]. Nevertheless, brain and testis have been the only organs in which DPP8/DPP9 activities precede over DPP4 activity [12,35,45,46]. Association of DPP8 and DPP9 with H-Ras suggests a functional role in signal transduction [41]. Furthermore, DPP8/DPP9 appears to be involved in T cell proliferation, thereby releasing IL-2 as well as macrophage activation causing activation of caspase 1 and induction of IL-1β [45,47–51]. However, the suggested cytotoxicity of DPP8/DPP9 inhibition is currently discussed controversially [52–56]. An increase of DPP8/DPP9 activity has been associated with asthma [44]. Extra-enzymatic functions of DPP8/DPP9 include cell adhesion, migration and apoptosis [57]. Interestingly, DPP8 and DPP9 are inactivated reversibly by H_2O_2 oxidation involving two cysteines in each monomer [58]. To date, there are no crystal structures of DPP8 and DPP9 available. Nevertheless, molecular modelling based on DPP4 and FAP crystal structures indicate similar overall structures comprised of β-propeller and α/β-hydrolase domains, with the active site being located at the interphase of the two domains. However, two loops and one helix of the propeller domain extending to the interphase cavity appear to play a role at the active site, thereby influencing substrate specificity and inhibitor binding [59,60]. Due to the shortest gene sizes, the lowest numbers of exons, the active site being located on one exon and their closest phylogenetic relationship with respect to prokaryotic members of the family, DPP8 and DPP9 are believed to be the ancestral genes of the DPP4 gene family [6].

DPP-like protein 1 (DPL1) and *DPP-like protein 2 (DPL2)*. DPP-like protein 1 (DPL1) and DPP-like protein 2 (DPL2) lack DPP4-like activity because of mutations at their active sites. Both of them are type II membrane-bound glycoproteins, suggested to interact with the

voltage-gated potassium channel Kv4 [4–6,61–63]. While DPL1 is expressed exclusively in the brain as two variants, i.e. a short and a long form, DPL2 is found in brain, pancreas and adrenal gland [5,62]. The long form DPL1-L is an 859 amino acid protein with a molecular weight of 97 kDa, whereas the short form, DPL1-S, consists of 803 amino acids with a reported molecular weight of 91 kDa [4,6]. The human gene localization is 7q36.1–q36.2. DPL1 is associated with amyotrophic lateral sclerosis, familial idiopathic ventricular fibrillation, spinal muscular atrophy and neuroleptic-induced tardive dyskinesia, whereas DPL2 is linked with asthma [44,61,64–68]. The crystal structures of DPL1 and DPL2, respectively, resemble that of DPP4 [63,69]. DPL2, better known as DPP10, is a type II membrane protein with a dimeric structure, comprised of alternative splice variants. The long form is expressed as a 796 amino acid protein with a molecular weight of 97 kDa. The human gene localization is 2q14.1 [5]. Table 1 summarises the properties of the DPP4 gene family members.

Non-related DPP4-like enzymes

In addition, enzymes structurally unrelated to the DPP4 gene family have been reported to display DPP4 activity [70]. These include DPP2, EC3.4.14.2 of SC clan 28, attractin and *N*-acetyl alpha-linked acidic dipeptidases (NAALADases I, NAALADases II and NAALADases L) from the metalloprotease clan MH, family M28B [70]. However, detailed kinetic analysis of expressed and purified NAALADase I did not reveal any DPP4-like activity [71]. Similarly, the DPP4-like activity of attractin in the serum had been discussed controversially for several years, but was later disproved [72–74].

DPP2, was found to be identical with quiescent proline cell dipeptidase (QPP) and dipeptidyl peptidase 7 (DPP7), based on genetic homology and kinetic parameters [75,76]. The soluble serine protease contains a proform and has a length of 492 amino acids with a molecular weight of 58 kDa [77,78]. Glycosylation and dimerization are required for the catalytic activity and the latter occurs via a leucine zipper motif, which is novel for proteases [79]. The homodimer is located in cellular vesicles that are distinct from lysosomes and secretion is regulated by an increased Ca^{2+} flux [77]. Using chromogenic substrates, DPP2 displays post-proline dipeptidyl aminopeptidase activity similar to DPP4, however, over a broad pH range with an acidic to neutral pH optimum [76,78,80]. While DPP2 hydrolyses tripeptides readily, its activity decreases rapidly with increasing chain length of peptide. Thus, it was shown to cleave only fragments of substance $P_{1–4}$, bradykinin$_{1–3}$ or bradykinin$_{1–5}$; however, we and others found none of the DPP4 substrates to be cleaved by DPP2 [12,80,81]. DPP2 has been reported to be involved in apoptosis, as a decrease of DPP2 activity caused cells to exit their G_0-phase in quiescent lymphocytes and fibroblasts, resulting in an induction of apoptosis by up-regulation of p53 and c-Myc as well as a down-regulation of Blc-2 [77,82]. Furthermore, DPP2 was found to be essential for maintaining the cell quiescence of lymphocytes, in which the transcription factors Kruppel-like factor (KLF2) and transducer of ERBB2, 1 (TOB1) regulate the expression of DPP2 [83]. Nevertheless, another study reports participation in necrosis rather than apoptosis [84]. DPP2 is distributed ubiquitously, with high expression in kidney, brain, testis, heart, resting lymphocytes and differentiated macrophages [75,78,84,85]. As it was thought previously to be a lysosomal enzyme, its physiological function to date is unknown. However, altered serum activities of DPP2 have been associated with various pathogenic conditions, such as Sjögren syndrome, rheumatoid arthritis (RA), lupus erythematosus, various cancers and Parkinson disease [78]. $DPP2^{-/-}$ and constitutive DPP2 knock-down (kd) are embryonic-lethal; however, conditional neurogenin 3-specific DPP2 knockdown mice revealed a phenotype with impaired glucose tolerance, insulin resistance and visceral obesity [86]. NGN3 is expressed in all precursors of the enteroendocrine cells and in the pancreas as well as discrete regions of the hypothalamus and brain stem [86]. Interestingly, ADA was discovered to also bind to DPP2, although with an order of magnitude lower compared to DPP4 [77]. The human gene localization is 9q34.3. As DPP2 also belongs to the SC clan, its order of catalytic residues is Ser, Asp and His, located in an α/β hydrolase fold, as summarized in Table 1. Recently, the crystal structure of DPP2 was deposited in the Protein Data Bank as pdb 3JYH, revealing an α/β-hydrolase domain as well as a novel helical structural domain (SKS) domain, comprised of 5 α-helices arranged in a helix bundle fold, capping the active site [87]. An insertion from the SKS domain to the active site results in steric hindrance of larger substrates and contains Asp_{334} for anchoring the *N*-terminus of the peptide substrate. Prolycarboxy peptidase, also belonging to the S28 family, displays a similar overall structure, whereas the members of the DPP4 gene family are made up of a propeller and an α/β-hydrolase domain. The propeller has an open architecture and contains eight blades, each made up of four anti-parallel β-sheets [87].

Although all these enzymes described above display DPP4-like activity or are structural homologues (Table 1), they exhibit distinct features with respect to cellular compartmentation and glycosylation, as illustrated in Fig. 1. Furthermore, DPP4 also internalizes upon association with binding partners such as CXCR 4 and M6P/IGFII, recycling of terminal carbohydrates and assembled to lipid rafts [8,9]

DASH proteins in immune cells

In addition to DPP4, DPP8, DPP9 and DPP2 were also found to be expressed on leucocytes, yet fulfilling different functions, as illustrated in Fig. 2 [35,47,48,51,82,88,89].

Table 1. Summary of the DASH-proteins comprised of the human *DPP4*-gene family [139] and DPP2 [5–7,36].

	Clan SC						
	Family S9b (DPP4-gene family)						S28
	DPP4	FAP	DPL1	DPL2	DPP8	DPP9	DPP2
Synonyms	DPIV, DPPIV, DAP IV, CD26, ADAbp, Gp108, Gp110, Hep105	Seprase, anti-plasmin cleaving enzyme, APCE	DP 6, DPP 6, DPPX-S, DPPX-L, DPPX	DP 10, DPP 10, DPP Y	DPP 8	DPP 9	DP II, DPP7, QPP
EC number	3.4.14.5	3.4.21.B28	–	–	–	–	3.4.14.2
Merops	S09.003	S09.007	S09.973	S09.974	S09.018	S09.019	S28.002
Accession	M80536	U09278	M96859 M96860	AY172659	AF221634	AF452102	AF154502
Gene location	2q24.3	2q23	7q36.2–3	2q12.3–2q14.2	15q22	19p13.3	9q34.3
Gene size (kb)	81.8	72.8	659	535	71	48.7	2.85
# Exons	26	26	26	26	20	22	13
Promoter	TATA-less, GC rich	?	?	?	TATA-less, GC rich	?	?
Regulation of expression	Tissue-specific HNFα/β Interferons α/β/γ RA, cytokines	?	?	?	?	?	KLF2, TOB1
# Amino acids	766	760	803/859	796	882	863	492
Mr (kDa)	110	97	115/120	91	100	98	54.3
Proform	–	–	–	–	–	–	yes (SP)
Transmembrane	✓	✓	✓	✓	–	–	–
Cysteines	12	13	11	9	12	17	8
# Glycosylation	9 (2–9)*	5	7	10	-	2	6
Glycosylation	N/O	N	N	N	-	N	N
Catalytic triad	✓	✓	–	–	✓	✓	✓
Intron at catalytic S	✓	✓	–	–	–	–	✓
Activity	DPP4	DPP4 + gelatinase	Unknown	Unknown	DPP4	DPP4	DPP4 (acidic pH)
Quaternary structure	dimer/tetramer	Dimer	Dimer	Dimer	Dimer	Dimer	Dimer
Activity needs dimer	✓	✓	–	–	–	–	✓
Crystal structure	✓	✓	✓	✓	–	–	✓
Subcellular location	Membrane type II/ soluble	Membrane type II/soluble	Membrane type II	Membrane type II	Cytosolic	Cytosolic/ Nuclear	Secretory vesicles Lysosomes

Table 1. *Continued*

	Clan SC						
	Family S9b (DPP4-gene family)						S28
	DPP4	FAP	DPL1	DPL2	DPP8	DPP9	DPP2
Binding partners	ADA, CD45, Cav-1 Collagen, Glypican-3, PgR, CARMA-1, HIV-TAT, CXCR-4, M6P/IGFII, FAP	$\alpha_3\beta_1$ Integrins $\alpha_5\beta_1$ Integrins Annexin 2 uPA-R, DPP4	A-type K(+) channel (Kv4.2)	A-type K(+) channel (Kv4.2)	H-Ras	H-Ras	ADA
Distribution	Ubiquitous Kidney > small intestine > lung	Activated-Fibroblasts Activated-Stellate cells	Brain	Brain, pancreas, adrenal gland	Ubiquitous Reproductive Organs, brain	Ubiquitous Heart, liver Skeletal, brain, muscle, testis	Ubiquitous kidney, testis,brain
Knock-out	✓	✓	✓	–	–	✓	✓
Transgenic	✓	–	–	–	–	–	–
Leucocytes	⇑ act.# T cells, ⇑ act.# B cells ⇑ act.# NK cells ⇑ act.# Macrophages	–	–	–	⇑ Peripheral Leucocytes Lymphocytes ≈ Monocytes	⇑ Peripheral leucocytes Lymphocytes ≈ Monocytes	⇑ Macrophages Resting Lymphocytes
Known physiological functions	Cell adhesion, Immune response, tumour progression, Psychoneuro-endo-crine function, metabolism	Cell growth regulation, Tumour progression, wound-healing Fibrogenesis	Embryonic development, Signal transduction, regulation of synaptic plasticity, K^+ channel	Signal transduction, Regulation of synapticplasticity, K^+ channel	T-cellproliferation? Signal Transduction Cell-survival Cell-proliferation	Antigen presenting Signal transduction Cell-survival Cell-proliferation	Neuroprotection? Maintaining lymphocyte quiescence
Pathological role	Diabetes Cancer MS, AIDS Anxiety	Cancer, Liver Cirrhosis Arthritis Pulmonary fibrosis	Neurodegeneration Progressive spinal muscular atrophy	Asthma Amyotrophic lateral sclerosis (ALS)	Breast -, ovary cancer	Prostate cancer	Apoptosis of leucocytes Neuroprotection diabetes
References	[6–8,13,59,140,141]	[6,7,13,21–23,28,94]	[4,6,7,44,63–66,133]	[5–7,61,62,69]	[2,6,7,13,31,33–35,94]	[6,7,13,35,36,38–41]	[6,7,75–78,80–86,117]

(2–9)* = Number of glycosylation sites being occupied in the crystal structures; # = activated. ADA = adenosine deaminase.

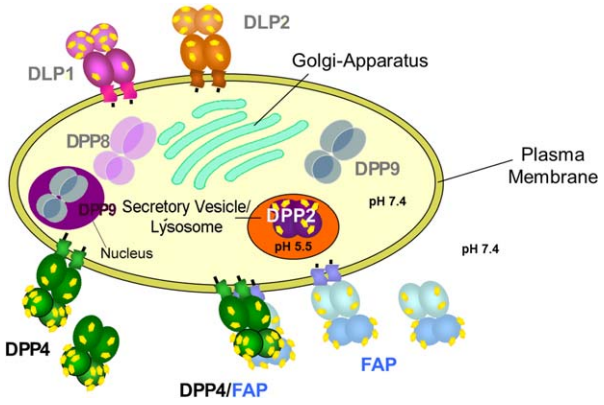

Fig. 1. Cellular compartmentation of dipeptidyl peptidase (DPP)4-like enzymes. DPP4 and fibroblast activation protein alpha (FAP) are located either as homodimers or heteromeric complex on the plasma membrane or shedded into the serum. DPP2, a homodimer, is distributed as a zymogen in secretory vesicles or lysosome. DPP8 and DPP9 are also homodimers and located cytosolically. ⬟, glycosylation.

DPP2 plays a vital role in quiescence of resting lymphocytes and its inhibition leads to apoptosis [82,90]. The activity of DPP8 and DPP9 is required for T cell proliferation and is IL-2-dependent [40,48,49,52,90]. DPP8 is up-regulated upon T cell activation, whereas DPP9 plays a role in antigen trimming for antigen presentation [2,39,40]. DPP4/CD26 is involved in T cell activation, T cell signalling and T cell differentiation due to its interactions with ADA, CD45, caveolin-1, CARMA-1 and M6P/IGFII-R [9]. These processes are regulated by the cytokines IL-2, IL-6, IL-10, IL-12, IL-17, IL-29, IFN-γ and TGF-β, as well as compartmentation of DPP4/CD26 to either lipid rafts or internalization [8,9,48,88,89,91–93]. Furthermore, post-translation modification of DPP4/CD26 such as sialylation also appears to influence compartmentation and/or the interactions with its binding partners [9,86,87,90]. Generally, expression of DPP4/CD26 is up-regulated in T helper type 1 (Th1) and Th17 cells, but not in Th2 cells. However, comparing CD28 *versus* CD26 co-stimulation of CD3 mediated T cell activation, CD26 co-stimulation was found to induce production of IL-10 preferentially in human $CD4^+$ T cells mediated via nuclear factor of activated T cells (NFAT) and rapidly accelerated fibrosarcoma–mitogen-activated protein kinase–extracellular signal-regulated kinase (Raf–MEK–ERK) pathways, as well as high levels of early growth response 2 (EGR2) mediated possibly via NFAT and activator protein 1 (AP-1)-signalling. Furthermore, CD26-mediated co-stimulation of $CD4^+$ T cells induced greater lymphocyte-activation gene 3 (LAG3) expression than CD28-mediated co-stimulation [92]. Whether or not the other DASH proteins contribute to the overall DPP4-like activity in Th2 cells such DPP8 still needs to be investigated [2,7,13,51,88,94,95]. In addition, DPP4/CD26 is expressed highly on the $CD45RO^+$ $CD29^+$ memory T helper subset $CD26^{bright}$ $CD4^+$, which responds to recall antigens, induces B cell immunoglobulin (Ig)G synthesis and activates cytotoxic T cells [7,8,13,51,94,96]. CD26/DPP4 also plays a role in chronic pulmonary graft-*versus*-host disease with up-regulation of IL-26, involving CD26 and caveolin-1 interactions [91]. Furthermore, DPP4 is up-regulated in activated natural killer (NK) cells, B cells, eosinophils and macrophages [9,13,44,45,51,94]. None the less, DPP8, DPP9 and DPP2 are also expressed on macrophages and DPP2 has been detected additionally on mast cells [13,35,40,47,51,78,85]. These differentiated leucocytes regulate the expression of the DASH proteins in/on endothelial, fibroblast and epithelial cells via their cytokines, thereby influencing physiological and pathophysiological processes such as vasoconstrictions, vasodilation, angiogenesis, transendothelial migration of lymphocytes, hypothalamic–pituitary–adrenal (HPA) stress axis, wound-healing, arthritis, cirrhosis, cancer, colitis, inflammatory bowel disease (IBD) and asthma, as illustrated in Fig. 2 [6,7,9,13,21,29,35,40,44,51,94,97–100]. Substrates of DPP4 and/or DPP8/DPP9 may also be involved in these scenarios, such as the chemokines regulated upon activation normal T cell expressed and secreted (RANTES), SDF-α and eotaxin, the neuropeptides NPY, SP, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP), as well as the peptide hormones GLP-2 and GLP-1 [7–9,11,12,14,20,24,31,33,34,40,51,100].

Knock-out, deficient and transgenic DPP4-like animal models

DPP4-knock-out, -deficient and -transgenic animal models have been useful to elucidate the physiological role of DPP4-like enzymes and *in-vivo* substrates. The phenotypes of such animal models are summarised in Table 2. Thus, $DPP4^{(-/-)}$ mice have provided evidence regarding the important role of DPP4 in the incretin metabolism of the insulinotrophic peptides GLP-1 and GIP. Additional increased energy expenditure and decreased food intake make DPP4 inhibitors a favourite pharmaceutical target compared to other known anti-diabetic drugs [101,102]. Furthermore, behavioural studies point to a possible negative involvement of DPP4 in stress-related behaviour, due probably to the modulation and/or inactivation of neuropeptide substrates, therefore identifying DPP4 as a potential pharmaceutical target in stress-related diseases [102]. Intriguingly, $DPP4^{(-/-)}$ seemed to be vital with normal immune responses, although they showed altered cytokine secretion and antibody production upon mitogen stimulation in serum, a down-regulation of $CD4^+$ T cells as well as up-regulation of NK cells in the spleen and a marked decrease of peripheral blood $CD4^+$ NK T cells [103]. Two substrains of Fischer 344 rats, the F344/Crl(Ger/$DPP4^-$) and F344/CuCrj(Jpn/$DPP4^-$) lack endogenous DPP4 at protein levels, while F344/Crl

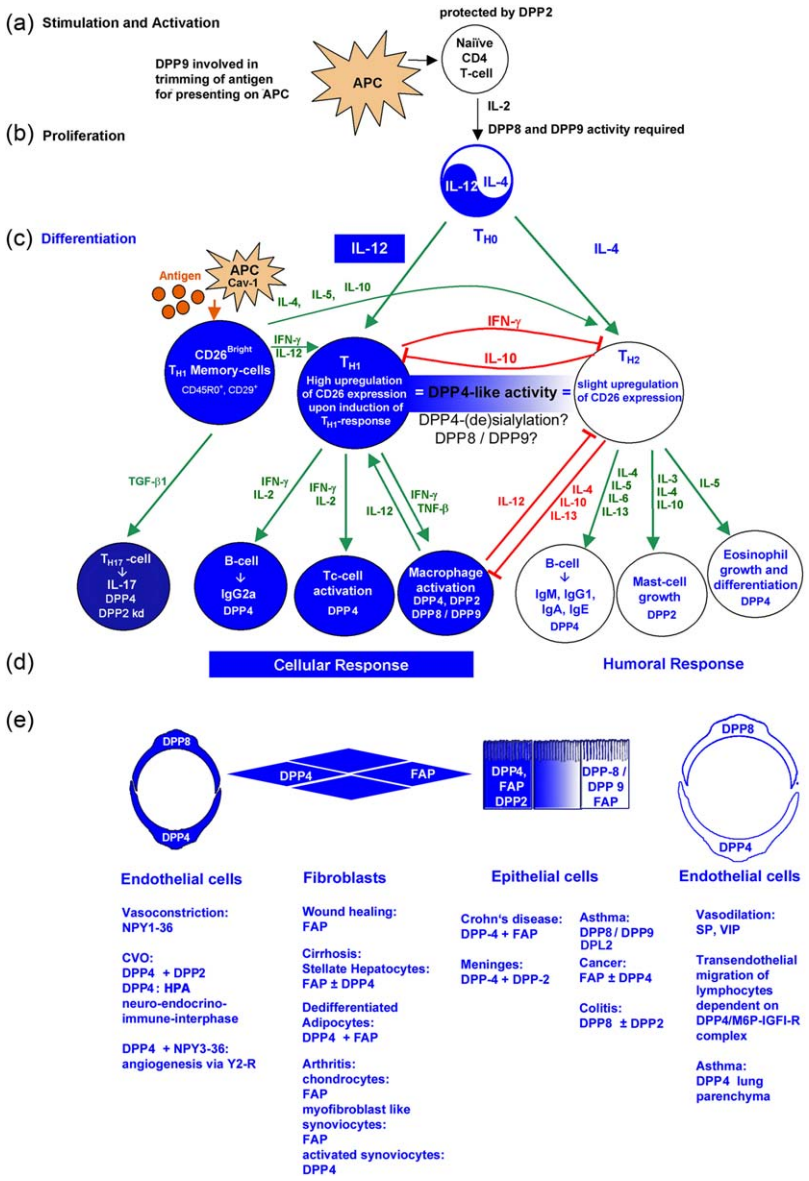

Fig. 2. Involvement of dipeptidyl peptidase (DP)4-like enzymes in T cell effector response. (a) Naive CD4 T cells are protected by DP2. Antigen-presenting cells (APC) stimulate and activate naive CD4 T cells. (b) Secretion of interleukin (IL)-2 results in clonal expansion, yielding T helper type 0 (Th0) cells. Enzymatic activities of DP8 and DP9 are required for this process. (c) Secretion of IL-12 and IL-4 results in the differentiation of Th1 and Th2 effector cells, respectively. Differentiation into Th1 cells initiates the up-regulation of DP4 expression. A small subset of $CD26^{bright}$ memory T cells already expresses high amounts of CD26. Upon antigen stimulation, $CD26^{bright}$ memory T cells augment the Th1 and Th2 response by secreting interferon (IFN)-γ, IL-12, IL-4, IL-5 and IL-10, respectively. Differentiation into Th2 cells results in only a slight up-regulation of CD26. The DP4 activity is equal in both T effector cells, due probably to specific DP4 isoforms or DP8 and/or DP9. (d) Differentiated T effector cells secrete specific cytokines that induce differentiation of leucocytes, resulting in cellular response by Th1 and humoral by Th2 effector cells. Leucocytes expressing DP4, DP2 or DP8/9 are indicated. Green arrow = stimulation; red arrow = suppression. [16,17,28,31,39–41,77–87,102,104].

(USA/$DPP4^+$) represents the wild-type [104]. Studies on these animals confirm the *in-vivo* role of DPP4 in diabetes, intestinotrophic peptide GLP-2 and stress-related behaviour, whereas isolated PMBC from mutant rats could be activated with mitogens similarly to the wild-type [105–109]. DPP4-deficient Fischer rats are used commonly as host animals for the transplantation of hepatocytes or stem cells obtained from wild-type animals, as DPP4 is a hepatic differentiation marker, and to distinguish between transplanted cells from the host cells [110]. Interestingly, mutant and wild-type F344 displayed the same phenotype with regard to arthritis, and non-selective DPP4-like inhibitors were able to suppress induced arthritis in both subspecies, implying the involvement of a DPP4-like enzyme other than DPP4, such as FAP [7,21,28,29,111]. Similarly, stimulation of neutrophils and erythrocytes from haematopoietic progenitor cells by a non-selective DPP4-like inhibitor was observed in both the mutant and wild-type F344 subspecies, again suggesting an involvement of another DPP4-like enzyme such as FAP, DPP8 or DPP9 [112]. A novel congenic DPP4-deficient DA.F344-$Dpp4^m$/SvH rat model confirmed the physiological role of DPP4 in glucose metabolism, immunology and stress-related diseases [113,114]. Like the DPP4-knock-out and -deficient animal models, transgenic mice with human CD26 displayed a normal immunological phenotype, although thymocyte proliferation as well as $CD4^+$ and $CD8^+$ T cell differentiation and viability was impaired, suggesting an important role of DPP4 in the T lymphocyte homeostasis in peripheral blood [115].

Intriguingly, $FAP^{-/-}$ mice displayed a phenotype with delayed wound-healing, but no increased susceptibility towards cancer [28]. However, $FAP^{(-/-)}$ hTNFtg mice revealed

Table 2. Summary of phenotypes obtained from DPP4-knock-out (k.o.), DPP4-deficient and DPP4-transgenic animal models as well as $FAP^{-/-}$ k.o., $DPP9^{S729A/S729A}$ gki., $DPL1^{-/-}$ k.o. and $DPP2^{-/-}$ k.o.mice [7,9,28,43,86,101–104,107,108,110–116,141–145].

Model	Investigation	Phenotype
DPP4 $^{(-/-)}$ mice	Protection from obesity + insulin resistance	⇑ Energy expenditure, ⇑ serum GLP-1, leptin, insulin, ⇑ Glucose tolerance ⇓ food intake $DPP4^{(-/-)} \approx$ DPP4-inhibition $\neq DPP4^{(+/+)} \Rightarrow$ DPP4 = target enzyme
	Immunology	Spleen: ⇓ $CD4^+$ T cells, ⇑ NK cells, stimulation (PWM): ⇓ IL-4,⇑ IL-10, IFN-γ Peripheral blood: ⇓ $CD4^+$ NK T lymphocytes After immunization (PWM): ⇓ IgG, IgG1, IgG2a, IgE, ⇓ IL-4, IL-2 + delayed IFN-γ $DPP4^{(-/-)} \approx DPP4^{(+/+)} \Rightarrow$ non-selective + DPP8/DPP9 inhibitors ⇓ T cell proliferation ⇒ DPP8/DPP9 involved
	Nociception	⇑ Plasma substance P, delayed pain response
	Cancer	$DPP4^{(-/-)} \approx DPP4^{(+/+)} \Rightarrow$ DPP4-like inhibitor ⇓ tumour cells
	Behaviour	⇓ Depression-like behaviour according to tail suspension and forced swim test $DPP4^{Mut} \neq DPP4^{WT} \Rightarrow$ DPP4 = target enzyme
	Disease	Experimental colitis: $DPP4^{(-/-)} \approx DPP4^{(+/+)} \Rightarrow$ DPP4-like inhibitor ⇒ intestinal adaptation
	Autoimmunity	MS: ⇓ TGF-β1, ⇓ Th1 immunity ⇑ clinical experimental autoimmune encephalomyelitis Arthritis: ⇑ serum SDF-α ⇑ arthritic inflammation
F344/Crl (Ger/$DPP4^-$) rats or F344/DuCrji ($DPP4^-$) rats	Protection from obesity +insulin resistance	Serum: * ⇑ GLP-1* ⇑ GIP, * ⇑ glucose tolerance, * ⇑ insulin after high fat- or glucose-diet, ⇓ $GLP\text{-}1_{13–36}$, ⇓ insulin resistance, ⇓ food intake after high fat diet, ⇓ weight gain $DPP4^{Mut} \approx$ DPP4-inhibition $\neq DPP4^{WT} \Rightarrow$ DPP4 = target enzyme
	Satiety	$DPP4^{Mut}$: ⇑ food intake + weight gain, ≈ postprandial [PYY] after 24 h fast due to $PYY_{1–36}$ $DPP4^{WT}$: peripheral administered PYY1-36 and PYY3-36: ⇓ food intake but not in $DPP4^{Mut}$ Short term DPP4-like inhibition: no anorectic effect of peripheral administered $PYY_{1–36}$
	Diseases	Asthma: peritracheal oedema: ⇑ oedema due to ACE inhibitors Glomerulonephritis: $DPP4^{Mut}$ = resistant to experimental induced glomerulonephritis Cholestasis: ⇑ serum DPP4-activity after induction of cholestasis in $DPP4^{WT}$ rats, no serum DPP4 activity in $DPP4^{Mut}$ Cancer: ⇓ $DPP4^{Mut}$ expression ⇒ ⇓ metastasis + cell adhesion Arthritis: $DPP4^{Mut} \approx DPP4^{WT} \Rightarrow$ ⇓ arthritic inflammation with DPP4 -like inhibitors ⇒ other DPP4-like enzymes involved in arthritis
	Immunology	Isolated PMBC from $DPP4^{WT}$ and $DPP4^{Mut}$ are able to be activated after mitogen activation ⇒ DPP4 may be involved but not necessary for T cell activation in rats Isolated splenic leucocytes from $DPP4^{WT}$ and $DPP4^{Mut}$ have the same proliferative response of *in-vitro* stimulation by T cells (Con A), B cells (LPS) and T + B cell (PWM) mitogens ⇒ DPP4 may be involved but not necessary for lymphocyte proliferation in rats. Altered age dependent leucocyte subset + thymic emigration pattern in $DPP4^{Mut}$ Asthma: $DPP4^{WT}$: ⇑ $CD4^+/CD26^+/CD25^+$ T cells recruitment in asthma induced lungs of rats ⇑ $CD26^+$: $CD26^-$ TCR cells ⇒ ⇑ IgE $DPP4^{Mut}$: ⇓ $CD4^+$ T cells ⇓ IgE, ⇑ recruitment of eosinophils, ⇓ recruitment of T cells Cancer: ⇓ NK cytotoxicity in $DPP4^{Mut}$ rats ⇒ DPP4 activity sustains NK cytotoxicity Haematopoiesis: $DPP4^{Mut} \approx DPP4^{WT} \Rightarrow$ DPP4-like inhibitor ⇑ neutrophils + erythrocytes from progenitor stem cells via G-CSF⇒DPP4-like enzyme involved in neutropenia and acute anaemia
	Nociception	⇑ Sensitivity to non-habituated pain stimuly and/or reduced stress-induced analgaesia
	Behaviour	⇓ Stress response in OF, SI, passive avoidance + EPM ⇓ motor activity $DPP4^{Mut} \neq DPP4^{WT} \Rightarrow$ DPP4 = target enzyme
	Small intestine	$DPP4^{Mut} \approx$ DPP4-inhibition ⇒⇑ GLP-2 + ⇑ bowel weight + resistance to gastrointestinal damage
	Assimilation of Pro in kidney + small intestine	⇑ Excretion proline containing peptides in urine; ⇓ weight in $DPP4^{Mut}$ after fed with gliadin Isolated brush border membranes from small intestines + kidney unable to hydrolyse proline containing peptides

Table 2. *Continued*

Model	Investigation	Phenotype
	Transplantation	Used as a model of stem cells/hepatocyte transplantation to distinguish between donor and recipient, DPP4 = HAM.4 = differentiation marker of hepatocytes
Da.F344-Dpp4^{m}/SvH	Protection from obesity +insulin resistance	Serum: ⇑ GLP-1 ⇑ Glucose tolerance ⇑ insulin after OGT ⇓ weight gain after high-calorie diet ⇓ islet size ⇒ $DPP4^{Mut} \neq DPP4^{WT} \Rightarrow$ DPP4 = target enzyme
	Lipid metabolism	⇑ Leptin signalling ⇑ bound leptin in plasma ⇓ free leptin in liver ⇓ triglycerides ⇓ alkaline phosphatase ⇓ aminotransferases
	Kidney + small intestine	Impaired assimilation of Pro in kidney and small intestine ⇓ weight in $DPP4^{Mut}$ after fed with gliadin
	Immunology	⇓ Lymphocytes ⇓ eosinophils ⇑ NK cells ⇑ B cells ⇓ NK cytoxicity ⇓ T cell proliferation ⇓ IL-6 Soluble DPP4 derived partially from bone marrow and not kidney according to transplantation studies
	Behaviour	⇓ ACTH ⇓ corticosterone ⇓ stress-induced hyperthermia ⇓ stress-induced analgaesia, ⇑ fear extinction, ⇑ NPY in CNS ⇓ stress response in hole board test, SI + EPM ⇒ $DPP4^{Mut} \neq DPP4^{WT} \Rightarrow$ DPP4 = target enzyme
huCD26tg mice	Immunology	⇓ Age-related thymus cellularity with impaired thymocyte proliferation ⇓ peripheral T cell pool with ⇑ apoptosis in $CD4^{+}$ and $CD8^{+}$ subpopulations
$FAP^{(-/-)}$ mice	General	Delayed wound-healing
$FAP^{(-/-)}$hTNFtg mice	Arthritis	Ameliorates cartilage destruction in inflammatory destructive arthritis
$DPP9^{S729A/S729A}$	General	GKI-$DPP9^{S729A/S729A}$ mice with inactive DP9 ⇒ neonatal lethal, $DPP9^{wt/S729A}$ ⇒ indistinguishable from wild-type
$DPL1^{(-/-)}$ mice	General	DPL $1^{(-/-)}$ ⇒ embryonic lethal, $DPL1^{(+/-)}$ ⇒ pigmentation defect
$DPP2^{-/-}$	General	Lethal
NGN3-specific $DPP2^{(-/-)}$ mice	Metabolism	⇑ Hyperinsulaemia, ⇑ glucose intolerance, ⇑ insulin resistance, ⇑ liver steatosis, ⇑ adipocytes resulting in visceral obesity

OP, open field test; SI, social-interaction test; EPM, elevated plus maze.

Red text: DPP4 inhibition results similar phenotype as $DPP4^{-/-}$ and both are different to wild-type $DPP^{+/+}$, confirming DPP4 as pharmaceutical target; green text: no differences between $DPP^{-/-}$ and $DPP^{+/+}$, but inhibition with non-selective DPP4-inhibitor shows pharmaceutical efficacy indicating that DASH-protein other than DPP4 is involved.

less cartilage degradation, but similar inflammation and bone erosion compared to wild-type hTNFtg mice [29].

$DPL1^{-/-}$ and $DPP2^{-/-}$ were found to be lethal, whereas $DPL1^{-/+}$ exhibited pigmentation defects and neurogenin-3 induced $DPP2^{-/-}$, a phenotype opposed to $DPP4^{-/-}$ with increased hyperinsulaemia, glucose intolerance, insulin resistance and liver steatosis [86,116]. Furthermore, mutant mice with knock-down of DPP2 in resting T cells (lck-DPP2 kd) led to differentiation into IL-17 releasing Th17 cells after *in-vivo* priming and *in-vitro* antigen-specific stimulation [117]. Homozygote gene knock-down $DPP9^{S729A/S729A}$ mice die shortly after birth, while heterozygote $DPP9^{wt/S729A}$ mice were morphologically indistinguishable from the wild-type. The results imply, on one hand, that enzymatic activity of DPP9 is essential for survival, and on the other hand that no other DASH protein is able to take over the role of DPP9, as none of them were up-regulated [43]. The physiological and pathophysiological role of DPP9 has been elucidated only recently, being involved in antigen presenting and the EGF signalling pathway [38–40]. This suggests that DPP9 plays a role in inflammation as well as cell proliferation and apoptosis [40,41].

DASH proteins as therapeutic targets

History and development of DPP inhibitors as well as modulators of DASH proteins

The first generation of DPP4 inhibitors were developed prior to the discovery of DPP8 and DPP9, and these include P32/98 (Ile-Thia), Lys[Z(NO_2)]-pyrrolidine, Lys[Z(NO_2)]-thiazolidide, Lys[Z(NO_2)]-piperidide, LAF-237 (vildagliptin), NVP-DP728, L-Pro-L-boroPro, Pro-Pro-diphenyl phosphonate esters, aminoacyl-pyrrolidine-2-nitriles, aminoacylpyrrolidides and aminoacyl-thiazolidides [7,8,118]. At this stage, data concerning selectivity were available only for DPP4 and DPP2. In retrospect, the importance of DPP4 selectivity over DPP2 has been elucidated by the opposing pathological roles of DPP2 in diabetes compared to DPP4 [86]. However, toxicological studies of the first-generation inhibitor P32/98 resulted in high toxicity with bloody diarrhoea, emesis and tenesmus in dogs and alopecia, thrombocytopenia, anaemia, enlarged spleen, multiple histological pathologies and mortality in rats. Subsequently, selective inhibitors were developed, and investigation of the above cytotoxic effects revealed the inhibition of DPP8 and DPP9 to

be responsible for the side effects [52]. Therefore, the second generation of anti-diabetic DPP4 inhibitors focused on the selectivity of the various DPP4-like enzymes [52,119–122]. Conversely, the requirement of selectivity is currently debated controversially, as some of the selective inhibitors against DPP8/9 were unable to enter the cell, suggesting that the cytotoxic effects observed were not due to inhibition of these cytosolic enzymes [53–56]. Given that DPP4, FAP, DPP8, DPP9 and even DPP2 have their highest sequence and structure similarities at the catalytic domain [6], uncompetitive inhibition at the propeller domain may be more specific for a particular DPP4-like enzyme. Molecular modelling of DPP8 and DPP9 revealed a P_2-loop at the propeller domain, containing F357 and R358, that seems to be unique to DPP8 and DPP9 and is suggested to influence substrate and inhibitor binding to the P_2-pocket [60].

In addition, administration of DPP4 inhibitors or anti-DPP4-monoclonal antibodies (mAb) have been demonstrated to improve additional disease conditions in various animal models, cell cultures or interfering with the interaction of binding partners, as summarised in Table 3 [123]. For example, a non-selective DPP4-like inhibitor, PT-100, had been in clinical trials II-III for various types of cancers based on its dual inhibitory action against FAP as well as DPP8 and/or DPP9, although it was discontinued in 2007 [30,49]. Dual inhibitor IP10.C8 against DPP4 and APN are currently being investigated for the treatment of autoimmune diseases such as psoriasis, multiple sclerosis (MS) and IBD [48,124]. Intriguingly, DPP4 and DPP2 again appear to have opposing roles regarding Th17. While DPP4 inhibition was shown to suppress the development of Th17 cell differentiation, knock-down of DPP2 in resting T cells of mutant mice led to differentiation into IL-17-releasing Th17 cells [48,125,126]. In fact, inhibition of DPP4 has been proposed for the treatment of the autoimmune disease diabetes type 1 by suppressing the pathogenic effects of Th1 and Th17 cells and up-regulating Th2 cells [127]. DPP2 selective inhibitor AX8819 was designed for a prognostic marker of B cell chronic leucocytic leukaemia, as well as a potential drug target to induce apoptosis in malignant B cells [126]. DASH inhibitors Lys[Z(NO_2)]-Thia, Lys[Z(NO_2)]-Pyr, TMC-2A, TSL-225, as well as FAP-specific inhibitor L-glutamyl L-boroproline, have been implemented for the treatment of arthritis [7,13,97,111,121,128]. FAP inhibitors have been developed to promote fibrinolysis [21]. Radioactive labelled anti-FAP-monoclonal antibodies have been applied for targeting tumour cells [129]. Furthermore, Pentostatin, an ADA inhibitor admitted by the Food and Drug Administration (FDA), was shown to reduce $CD26^+$ T lymphocytes preferentially [130]. Chronic administration of haloperidol increased the gene expression of DPL1 in mouse brains. Latter findings indicated an altered response of Kv4/DPP6 to long-term neuroleptic administration [66].

Role of DASH proteins in diseases

Cancer

DPP4 has been proposed as a biomarker for a variety of cancers, such as thyroid, colon, breast, prostate and malignant pleural mesothelioma as well as lymphoma, β cell chronic leucocytic leukaemia and T cell lymphoid malignancies [7–9,22,23,130,131]. In addition, expression of FAP has been investigated in several types of cancers as potential biomarkers for epithelial colon, gastric, intestinal, oesophageal, undifferentiated thyroid, lung, breast, ovarian and cervical cancers, meningioma, glioma and cutaneous melanoma, as well as aggressive fibromatosus [7,12,15,17]. In cancers and capillaries where DPP4 and FAP co-localize, they form a heteromeric complex with both enzymes still maintaining their activities. This complex protrudes at invadopodia, where gelatin is binding to DPP4 and being degraded by FAP, respectively. Truncation of NPY by DPP4 results in angiogenesis, whereas chemokines such as SDF-α are involved for migration and invasion. Thus, by means of their associations and substrate specificities, the DPP4/FAP heteromeric complex is responsible for tumour invasion, migration, metastasis and angiogenesis [6,7,13,22–24].

Furthermore, potential roles of DPP8 and DPP9 have been suggested in breast and ovarian cancer and an up-regulation of DPP9 has been detected in testicular tumours [35,94,132]. In addition, DPP9 was found to regulate cell survival and proliferation by inhibiting Akt activation involving the EGF signalling pathway. Moreover, DPP9 and DPP8 associate with H-Ras, a key signal molecule of the EGF receptor signalling pathway [41]. DPP8 and DPP9 have also been proposed to play a role in cell adhesion, migration and apoptosis [57].

Finally, DPP2 appears to be a prognostic biomarker and drug target for B cell chronic leucocytic leukaemia [126].

Asthma

Investigating the effects of airway inflammation in wild-type and DPP4-deficient rats, a significant increase of DPP4 enzymatic activity was found in the lung parenchyma as well as DPP8/DPP9 enzymatic activity in the bronchial epithelium. Furthermore, these enzymes also displayed elevated activities in bronchoalveolar lavage fluid. In addition, strong immunohistochemical staining was detected in bronchial epithelium and trachea for DPP8, DPP9 and DPL2, respectively. These results were also confirmed by elevated mRNA levels of DPP8, DPP9 and DPL2 in bronchial epithelium and trachea of asthmatic lungs. In contrast, increased staining of DPP4 and T cells was found in asthmatic lung parenchyma. Thus, the results revealed differential and site-specific expression of DASH proteins in lung as well as their up-regulation and functions in asthma [44]. In fact, DPL2 has been proposed for asthma susceptibility [133].

Table 3. Summary of disease conditions, being potential targets for DASH inhibition or modulated by anti-DPP4-mononuclear antibody (mAb) and/or anti-FAP-mAb [7,9,10,13,30,52,121,123,124,128,129,146–151].

Target disease	Effect of inhibitor	Development stage	Comments	Inhibitor/mAb
Type 2 diabetes	Inhibition of incretin deactivation, improvement of postprandially stimulated insulin secretion and glucose tolerance	FDA approved: Sitagliptin, Saxagliptin, Linagliptin EMEA: Vildagliptin Japan: Alogliptin Korea: Gemigliptin	Many pharmaceutical companies are targeting DPP4 inhibition	Sitagliptin (*Januvia*) Saxagliptin (*Onglyza*) Linagliptin (*Ondero*) Vildagliptin (*Galvus*) Alogliptin (*Nesina*)
Tumours	Prevents spreading of metastases, inhibits tumour migration and angiogenesis by inhibiting FAP	Phase III: FDA put on hold	Disapproved treatment on lung and pancreas cancer	Talabostat (PT-100)
	Inhibition of DPP8 + DPP9: ⇑ IL-β ⇒ ⇑ cytokines + ⇑ chemokines ⇒ ⇑ neutrophils, ⇑ T-lymphocytes, ⇑ macrophages	Phase II studies: Melanomas	Clinical trials for melanomas	(Val-boroPro) Discontinued
	Cell-specific suppression of stromal tumours expression high levels of FAP	Human Clinical Phase I Animal model	F19-antibody radio-labelled chemotherapy	FAP-mAbF19 Oral DNA vaccine against FAP
	Prevents spreading of metastases, inhibition of tumour migration and angiogenesis	Cell culture, animal models	Hetero-dimerization with FAP involved	DPP4-mAb 1F7 Pro-boroPro
	Anti-cancer effects and tumour cytotoxicity	Cell culture, animal models	Z-GP-Dox, an FAP-based doxorubicin prodrug	Z-GP-Dox with, systemically delivered via mixed nanomicelles
	Potent anti-tumour effects by increased levels of natural killer cells and DCs as well as making murine and human tumour cells more sensitive to antigen-specific CTL killing	Cell culture, animal models	Pan inhibitor of DASH	ARI-4175 in combination with a recombinant viral or dendritic cell (DC)-based tumour-cell vaccine
Multiple sclerosis	Suppression of EAE, involves Th17 cells	Animal experiments,human primary cell culture	Autoimmune disease	Lys[Z(NO_2)]-Pyr PETIR-001
Psoriasis	Reduction of keratinocyte hyperproliferation, involves Th17 cells	Cell culture, animal models, preclinical studies	Autoimmune disease	Lys[Z(NO_2)]-Thia Lys[Z(NO_2)]-Pyr P32/98 PETIR-001
Colitis	Suppression of GLP-2 inactivation	Cell culture, animal models	DPP4, DPP8 and DPP2 may be involved	P32/98 PETIR-001
IBD	Suppression of GLP-2 inactivation, involves Th17 cells	Cell culture, animal models	DPP4 involved	P32/98 PETIR-001
Rheumatoid arthritis	Suppression of symptoms via inhibition of DPP4 + FAP (L-glutamyl L-boroproline)	Animal models	DPP4 + DASH inhibition ⇒ PT of CD45 inhibition FAP involved	Lys[Z(NO_2)]-Thia Lys[Z(NO_2)]-Pyr TMC-2A TSL-225 L-glutamyl L-boroproline
Anxiety/stress	Suppression of NPY cleavage decreases anxiety	Effective in animal models (rats)	Suggested to mainly involve NPY + DPP4	P32/98

Table 3. *Continued*

Target disease	Effect of inhibitor	Development stage	Comments	Inhibitor/mAb
Psychosis/schizophrenia	DPP4 inhibitor blocks mescaline-induced scratching + amphetamine-induced hyperactivity Haloperidol ⇑ Kv4/DPP6 ⇒ ⇓ tardive dyskinesia	Effective in animal models (mice)	May also involve endomorphin-1/2 Haloperidol ⇑ Kv4/DPP-6	AMAC (DPP4) Haloperidol (DPP-6)
Graft rejection	Suppression of graft rejection	Animal experiments		Pro-Pro diphenylphosphonate ester (prodipine)
Autoimmune disease	General immunosuppressive effects, involves Th17 cells	Cell culture, animal models	High doses necessary Dual inhibition of DPP4 and AP N (PETIR-001)	Lys[Z(NO_2)]-Thia Lys[Z(NO_2)]-Pyr Ala-boroPro Pro-boroPro PETIR-001
Acne	Inhibitors suppressed proliferation and IL-2 production of *Propionibacterium acnes*-stimulated T cells *ex vivo* + ⇓ TGF-β	Cell culture	Dual inhibition of DPP4 and AP N (PETIR-001)	Lys[Z(NO_2)]-Thia Lys[Z(NO_2)]-Pyr PETIR-001
T cell lymphoid malignancies	Enhanced cell cycle arrest at the G_1-S checkpoint, resulting in increased p21 expression	Cell culture, animal models	Influences survival and *de-novo* tumour growth	DPP4-mAb 1F7 Diprotin A Val-Pyr
B-CCL	Prognostic marker + drug target	*Ex-vivo* apoptosis assay	DPP2 inhibition	AX8819
AIDS	Suppression of SDF-α cleavage, suppression of gp 120 and DPP4 interaction	Cell culture	Mechanism not fully understood	Phe-Pyrr-2-CN Arg(PMC)-Pyrr-2-CN
Fibrinolysis	Suppression of α-anti-plasmin of cleavage	*In-vivo* clotting	Involves FAP	Acetyl-Arg-(8-amino-3,6-dioxaoctanoic acid)-D-Ala-L-boroPro
Wound-healing	Promotion of wound-healing	Cell culture	Heteromeric complex of DPP4 with FAP involved	DPP4-mAbE26 DPP4-mAbE19 DPP4-mAbE3

Red text: inhibitor admitted or disapproved by a regulatory authority. PT, phosphatase activity.

Arthritis

Expression and activity of DPP4 in arthritis is up-regulated in peripheral blood T lymphocytes and reduced in serum and synoviocytes, respectively [134]. SDF-α, one of the best substrates of DPP4, plays an important role in the pathogenesis of arthritis. However, the regulatory mechanism of DPP4 and SDF-α in arthritis appears to be somewhat complex and has not yet been elucidated [8,134]. Different results were obtained from clinical and epidemiological studies with anti-diabetic DPP4 inhibitors regarding increased or lower risks of polyarthropathy and autoimmune diseases such as RA in patients with type 2 diabetes mellitus (T2DM) [135,136]. Implementing differential diagnostics of polyarthropathy other than RA, osteoarthritis and crystal-associated arthritis, as well as analysing various cytokines and chemokines, higher incidences of polyarthropathy, were found in patients treated with DPP4 inhibitors. Intriguingly, the polyarthropathy was associated with reduced levels of SDF-α in plasma from T2DM patients receiving DPP4 inhibitors. Following cessation of DPP4 inhibitors, the clinical symptoms of polyarthropathy resolved within 3 months and the plasma levels of SDF-α were restored [135]. In contrast, the risks of developing an autoimmune disease such as RA seems to be lower upon treatment with DPP4 inhibitors, according to epidemiological studies [136]. A single nucleotide polymorphism (SNP) within an intron of DPP4 has been identified recently as a novel risk locus of RA [137]. However, *in-vivo* studies with three different DASH inhibitors ameliorated disease symptoms in both wild-type as well as DPP4-deficient F344 rats, implying the involvement of additional DPP4-like enzymes [111]. Although FAP was found to be up-regulated in chondrocytes and myofibroblasts of synoviocyte-like cells from patients with osteoarthritis and RA, specific inhibition of FAP and DPP4 resulted in increased invasion of activated synoviocytes due to elevated SDF-α levels, ruling out at least DPP4 and FAP as pharmacological targets against arthritis [21,98,128]. In contrast, synovial fibroblasts (SF) of $FAP^{(-/-)}$ hTNFtg mice had a reduced cartilage adhesion capacity compared to hTNFtg SF *in vitro*. This indicates an unknown role of the FAP protein, but not its enzymatic activity in the attachment of SF to cartilage, promoting proteoglycan loss and subsequently cartilage degradation in chronic inflammatory arthritis [29]. Investigations of FAP expression in SF from patients with RA and osteoarthritis (OA) revealed elevated FAP levels in RA, thereby confirming FAP being involved in chronic inflammatory arthritis [29].

In contrast to the activity of DPP4 in serum and synovial fluids, DPP2 activity was found to be increased, although its function has not yet been elucidated [78,138].

IBD

IBD includes Crohn's disease and ulcerative colitis [9]. The involvement of DASH proteins has been elucidated based on $DPP^{-/-}$ mice and DPP4/DPP4-like inhibitors. In Crohn's disease, pharmacological inhibition of DPP4 by two different inhibitors reduced disease activity significantly due to elevated GLP-2, indicating DPP4 to be a pharmaceutical target in Crohn's disease. However, in colitis DPP4 inhibitors and $DPP4^{-/-}$ mice were less effective, suggesting the involvement of other DASH proteins such as DPP8 and DPP2 [97].

Conclusion

Taken together, DASH proteins play important roles in the immune system involving quiescence, proliferation, antigen-presenting, co-stimulation, T cell activation, signal transduction, differentiation and tissue modelling. Thus, they are involved in many pathophysiological processes and have therefore been proposed for potential biomarkers or even drug targets in various cancers and inflammatory diseases. However, they also pose the challenge of drug selectivity concerning other DASH members for better efficacy and/or avoidance of unwanted side effects. Hence, more knowledge is needed to disentangle the complex roles of DASH proteins in immunology.

Disclosure

The authors have no disclosures to declare.

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