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Review article

Overcoming the intestinal barrier: A look into targeting approaches for improved oral drug delivery systems

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ABSTRACT

Oral drug administration is one of the most preferred and simplest routes among both patients and formulation scientists. Nevertheless, orally delivery of some of the most widely used therapeutic agents (e.g., anticancer drugs, peptides, proteins and vaccines) is still a major challenge due to the limited oral bioavailability associated with them. The poor oral bioavailability of such drugs is attributed to one or many factors, such as poor aqueous solubility, poor permeability, and enzymatic degradation. Various technological strategies (such as permeation enhancers, prodrugs and nanocarriers) have been developed to enhance the bioavailability of these drugs after oral administration. Among the different approaches, advanced and innovative drug delivery systems, especially targeting-based strategies, have garnered tremendous attention. Furthermore, the presence of numerous types of cells and solute carrier transporters throughout the gastrointestinal tract represents numerous potential targeting sites for successful oral delivery that have not yet been exploited for their full potential. This review describes different targeting strategies towards different targeting sites in the gastrointestinal tract. Additionally, exciting improvements in oral drug delivery systems with different targeting strategies (e.g., M cells for oral vaccination and L cells for type 2 diabetes mellitus) are also discussed.

1. Introduction

The oral route of drug administration is one of the most preferred routes of administration because of higher patient compliance, less expensive manufacturing costs and ease of administration. Furthermore, oral administration is used for both local and systemic delivery of a wide range of drug molecules, from small molecule drugs to large biomacromolecules. Despite the abovementioned advantageous features, oral delivery faces several limitations, such as poor solubility, poor permeability, fast degradation in the gastrointestinal tract, and an inability to penetrate the protective mucosal barrier [1]. A number of approaches have been studied to overcome the limitations of oral delivery of therapeutics. Among these approaches, targeting-based drug delivery systems have been designed and developed to overcome the shortcomings of newly discovered drug molecules and conventional formulations for delivery via the oral route. Targeted drug delivery systems (DDSs) reduce dose-related toxicity and improve therapeutic efficacy by augmenting the oral bioavailability of the drug molecules and by diminishing the minimum effective concentration of the drug. The targeted system achieves the aforementioned objectives by increasing the transport of the carrier/active compound to the targeted site and/or by enhancing the target to nontarget tissue ratio of the drug molecule [2]. Targeting is a very interesting approach to enhance oral delivery systems owing to the large variations along the gastrointestinal tract (GIT). Targeting different regions of the GIT can be achieved by exploiting its anatomical, histological, physiological, and biochemical features and combining the drug with advanced drug delivery systems or devices [3]. Some of the versatile physiological factors of the GIT include gastrointestinal pH differences, mucus thickness and GI transit time. Furthermore, the presence of numerous types of cells (e.g., enterocytes, Paneth cells, and L cells) and transporters interspersed throughout the GIT can be exploited. Such unique variations and features allow for the design of a broad array of targeted delivery systems, both passive and active.

Passive targeting is achieved by augmenting accumulation at the targeted site by exploiting pathophysiological features such as the temperature, pH, abnormal vasculature, or surface charge of the cells [4]. However, the random nature of targeting and its insufficient or unspecific characteristics seriously limit its application. To attain passive targeting, the properties of the system, such as charge, size, hydrophobicity and hydrophilicity, are altered [5]. On the other hand, in active targeting, specific ligands are linked on the surface of the DDS, which directs the whole system to the specific tissue/site that expresses ligand-specific biomarkers. Entire molecules or fragments of antibodies,

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vitamins, glycoproteins, folate, peptides and carbohydrates are some commonly used ligands for active targeting [6]. The ligands interact with the receptors present on the target site surface, thus resulting in the accumulation of the delivery system at the target site of action from where they either can act superficially or be internalized [7]. The necessity for targeted systems is remarkable in the case of anticancer drugs, where exposure to cytotoxic agents can damage healthy tissues. Other applications include targeting specific absorption sites, such as the small intestine, or targeting specific regions, such as inflamed colonic regions, in inflammatory bowel disease patients [8].

In humans, the GIT is a highly absorptive region that extends from the oral cavity to the anal canal, covering a total surface area of 250–400 m^2 [9]. There are four main types of gastrointestinal epithelial cells, which originate from stem cell populations in the crypt zone. These cells differentiate into absorptive (enterocyte/colonocyte), mucus secretory (goblet cells), hormone-secreting (enteroendocrine cells) or defensive peptide-secreting (Paneth cells). The specific physiological features of these gastrointestinal cells are described in detail in the next subsections, along with specific targeting strategies for each type of cell.

The present review overviews the latest and/or most commonly used strategies to target the different cell types and transporters present in the GIT.

2. Targeted intestinal cell drug delivery systems

There are a wide variety of cells comprising the GI epithelia that present different characteristics and receptors, especially in the small intestine (Fig. 1). Enterocytes are the most commonly found cells in the small intestine and are polarized absorptive columnar epithelial cells. The apical side of columnar epithelial cells is covered by a microvilli brush border and a carbohydrate glycocalyx. Mucus-secreting goblet cells are the second most commonly found cells (approximately 16 %) in the small intestine. The mucin secreted from these cells comprises the protective mucus layer that envelopes and protects the intestinal epithelia. M cells constitute less than 1 % of the total cells in the intestine; however, they play a vital role in the uptake of antigens and microorganisms. M cells are found in Peyer's patches where the mucus layer is absent, thus allowing closer interactions of the antigens and microorganisms with the intestinal epithelia. Dendritic cells (DCs) are responsible for maintaining immune environment homeostasis in the intestine by linking humoral and cellular immune responses [10]. Paneth cells are secretory cells and are found at the base of the crypts of Lieberkuhn. The main secretions of these cells are antimicrobial proteins and peptides [11]. The small intestine also contains enteroendocrine cells interspersed among the other epithelial cells. Although very low in number, enteroendocrine cells are responsible for secreting hormones with very important digestive functions. These cells are also involved in moderating communication between the central and enteric endocrine systems.

Targeting of intestinal cells is initiated by well-known ligand-receptor recognition. For this purpose, surface functionalized DDSs or ligand-grafted drugs (prodrugs) are required. Such targeted systems are designed to increase the uptake of the delivery system and encapsulated/grafted drugs at the target site. In the following subsections, the utilization of ligands for each type of epithelial cell will be discussed.

2.1. Enterocyte targeting

Enterocytes are the most prevalent type of cells in the intestinal epithelia and thus are often targeted to maximize the delivery of drugs and their absorption into systemic circulation. The entry of targeted DDSs into enterocytes normally occurs via receptor-mediated endocytosis. Endocytosis is initiated by the interaction between the ligand molecules on the surface of the DDS and the receptor molecules present on the epithelial cell surface, which results in the internalization of the complex [12]. Enterocytes are polarized cells with a columnar shape that have receptors for various compounds present on their apical surface (such as vitamins, transferrin and hormones) [13]. Therefore, taking advantage of such receptors is one of the most studied approaches for enterocyte targeting to enhance the efficiency of oral drug delivery. These ligands generally include vitamins [14–16], transferrin [17], lectins [18,19] and monoclonal antibody fragments [20], as well as oligopeptides [21,22]. Ligands that have been exploited to target enterocytes are summarized in Table 1.

2.1.1. Vitamins

Vitamins are widely used as ligands to decorate functional systems owing to their good safety profile, stability and easy tunability [23]. The ligands of vitamins used in enterocyte targeting include vitamin B_{12} (B_{12}), biotin (vitamin B_7), folic acid (vitamin B_9) and thiamine, of which B_{12} and folic acid are the most studied ligands for oral targeted delivery. B_{12}, a water-soluble vitamin, is a complex molecule that has a 'corrin ring' and a nucleotide [24]. B_{12} is orally absorbed after it binds to haptocorrin (a salivary enzyme), which protects and transports B_{12} to the small intestine. In the small intestine, B_{12} binds to an intrinsic factor forming the B_{12}-intrinsic factor complex, which interacts with receptors mainly expressed on enterocytes in the iliac region [25]. Various poorly absorbed drugs, proteins and peptides (such as insulin), among other molecules, have greatly increased their oral bioavailability by using B_{12} as a ligand for targeted delivery. B_{12}-decorated 70 kDa dextran nanoparticles (NPs) containing 4 % w/w insulin demonstrated a significant increase in pharmacological availability (2.6-fold higher) compared to nontargeted NPs [16]. The effectiveness of the B_{12}-NP conjugates was shown to be further improved by altering the different parameters of cross-linking [26]. The authors demonstrated that using an amino alkyl B_{12} derivative resulted in a significant hypoglycemic effect and extended (54 h) antidiabetic effects. Furthermore, the pharmacological availability of insulin also increased up to 29.4 % [26]. Notably, the cellular internalization of the soluble B_{12}-intrinsic factor complex is mediated via clathrin-coated vesicles followed by an endosomal stage that results in the degradation of intrinsic factors and the recycling of B_{12}. However, B_{12}-modified NPs disrupt the innate biological pathway of soluble B_{12}, changing it from clathrin-mediated endocytosis to the caveolae-mediated endocytic pathway (avoiding the endolysosomal pathway [27]. Various B_{12}-protein/peptide bioconjugates, for example, B_{12}-insulin [28], B_{12}-human peptide YY (B_{12}-hPYY) [29] and B_{12}-luteinizing-hormone-releasing hormone antagonists (B_{12}-LHRH) [30], have increased their intestinal absorption via the B_{12} pathway. B_{12}-coupled nanocarriers and bioconjugates have shown significant increases in the absorption of drugs; however, the potential applications of B_{12} are compromised by its relatively slower uptake compared with other vitamins and its limited availability of absorption sites (mainly in the distal ileum) [31].

Folic acid, vitamin B_9, is absorbed via a saturable pH- and sodium ion-dependent route and metabolic inhibitor-sensitive pathway [32]. Unlike B_{12}, folic acid receptors are present in sufficient quantities and fortify their response by improving the uptake and transport of bioactive molecules or vesicular systems across the GIT. It has been reported that various poorly absorbed drugs, such as protein drugs (insulin), antibiotics (vancomycin), and anticancer drugs (docetaxel and paclitaxel), have shown significant enhancements in their bioavailability via specific folic acid receptor targeting [33–37]. Similarly, biotin, also known as vitamin B_7, is transported not only through sodium-dependent multivitamin transporter (SMVT) but also via the biotin receptor, which are both distributed throughout the small intestine [38]. Zhang et al. developed biotin-modified liposomes for oral insulin delivery via specific clathrin-mediated endocytosis. The biotinylated liposomes demonstrated a significant increase in oral insulin bioavailability (8.3 %) compared with classical liposomes (3.3 %) [39,40]. Thiamine, as a vitamin ligand, has also been investigated to target the intestinal

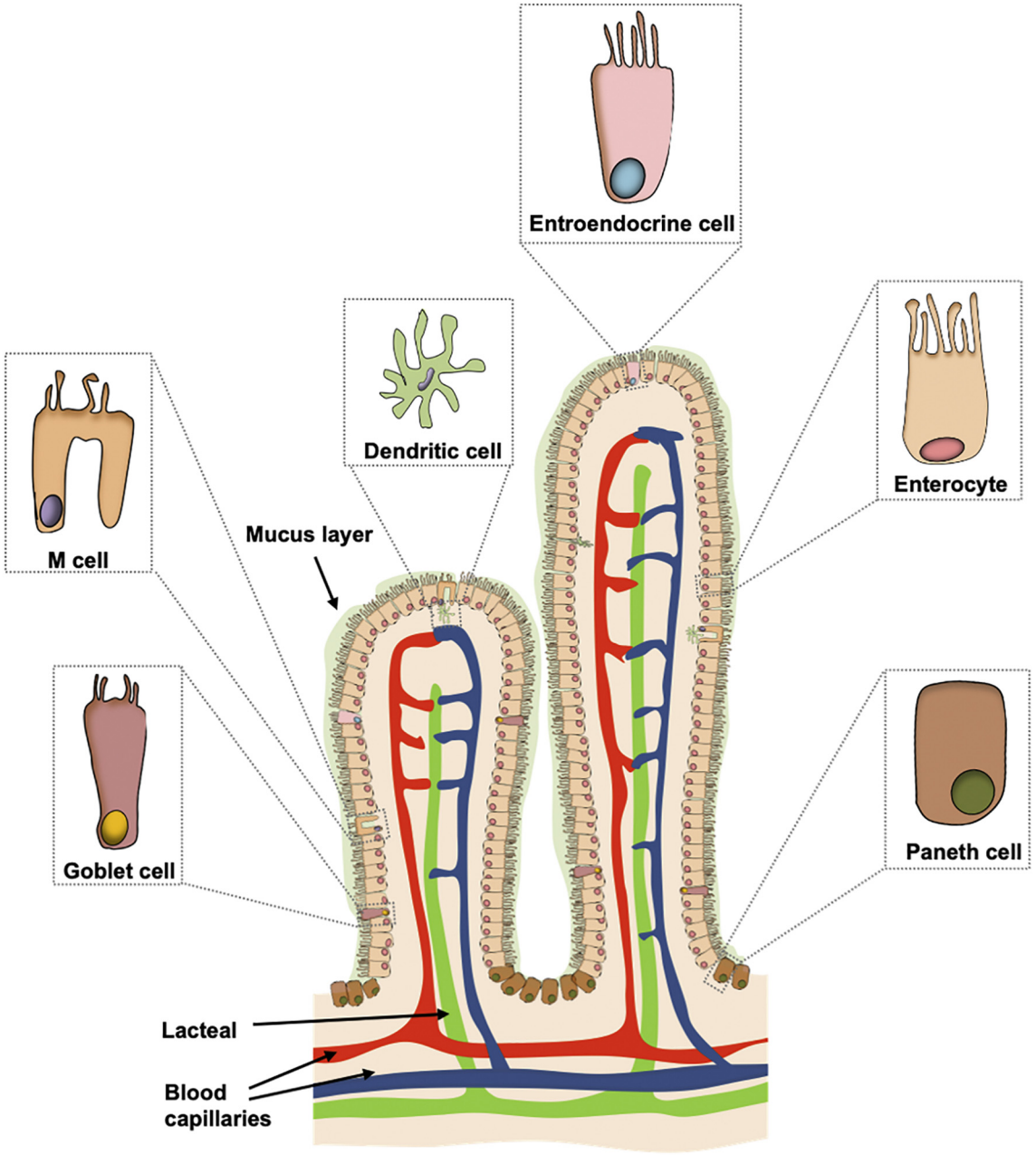

Fig. 1. Schematic representation of different intestinal cells present in the intestinal epithelium.

epithelia, owing to its outstanding features, such as high absorption in the human intestine, safety and affordability [15]. Salman et al. developed a Gantrez® AN NP (TNP), a thiamine-conjugated poly(methyl vinyl ether-co-maleic anhydride), for oral ovalbumin delivery (OVA; OVA-TNP) to male Wistar rats *in vivo*. The rats were orally immunized with OVA-TNP, which resulted in stronger and more balanced immune response profiles compared to OVA-loaded noncoated nanoparticles. This improved efficacy was attributed to the higher bioadhesion of the coated nanoparticles resulting in colonization in the lower regions of the gastrointestinal tract [15]. This was the first time that thiamine was used as a ligand for the oral delivery of an antigen for vaccination in an animal model.

2.1.2. Neonatal Fc receptors

Recently, the use of a membrane protein, the neonatal Fc receptor (FcRn), to develop intestinal targeted delivery systems has gained momentum. The increasing use of these receptors to improve the oral efficacy of drug molecules is mainly due to their wide availability on the surface of different types of cells, such as those of epithelial, endothelial, and myeloid lineages [41]. Furthermore, these FcRns are expressed from the neonatal stage into adulthood in the human intestine. They function as a receptor for both immunoglobulin G (IgG) and albumin, binding in the acidic intracellular compartment and protecting IgG and albumin from intracellular enzymatic breakdown [41]. These receptor-ligand complexes are then transcytosed by endosomes and released in the extracellular space at physiological pH, where the receptors are recycled [42] [43]. Thus, using the Fc portion of IgG or albumin as a targeting ligand is a promising approach to augment the passage of drugs/nanocarriers across the duodenum and jejunum [44,45]. Pridgen and coworkers were the first to demonstrate an FcRn-targeted DDS for oral administration [46]. They developed poly(lactic acid)–poly(ethylene glycol) (PEG-PLA) block copolymer-based nanoparticles, with the Fc portion of IgG chemically linked to the surface of the nanoparticles. Insulin was loaded into these nanoparticles (insNP-

Table 1
Examples of active enterocyte targeting in oral drug delivery.

Ligands	Cargo	Drug delivery systems	References
Albumin	Insulin	Albumin-porous silicon NPs	[44]
	Insulin	Chitosan/albumin-alginate/dextran sulfate NPs	[48]
Biotin (Vitamin B_7)	Insulin	Biotin-liposomes	[39,40]
Fc	Exenatide	Fc-PEG-PLGA NPs	[75]
	Follicle stimulating hormone (FSH)	Fc-FSH prodrug	[76]
	Insulin	Fc-PEG-PLA NPs	[46]
	Glucagon-like peptide-1 (GLP-1)	Fc-porous silicon NPs	[77]
	GLP-1 gene	Human IgG1 (hIgG1)-Fc-Arg/pDNA complexes	[45]
	SP141	Fc-PEG-PCL NPs	[14]
Folic acid (Vitamin B_9)	Cefotaxime	Folic acid-liposomes	[35]
	Docetaxel (DTX)	Folic acid-thiolated chitosan NPs	[36]
		Folic acid/bornel PLGA NPs	[78]
	Insulin	Folic acid-PEG-PLGA NPs	[79]
	Insulin	PLGA/folic acid-Chitosan NPs	[80]
	Insulin	Folic acid-liposomes	[33]
	Oxaprozin	Folic acid-PEG2k-NLCs	[81]
	Paclitaxel	Folic acid-PLGA NPs	[37]
	Paclitaxel	Folic acid-Pluronic F127-micelles	[82]
	Texas Red-Dextran 3000 (TR-dex)	Folic acid-PEO-liposomes	[32]
	Vancomycin	Folic acid-liposomes	[34]
FQSIYPpIK (FQS)	Insulin	FQS-TMC NPs	[21,69]
HAIYPRH (7peptide)	coumarin-6 (C6)	7peptide-PEG-b-PCL micelles	[22,83]
ICAM-1	–	ICMA-1-polystyrene nanocarriers	[84–86]
Thiamine	Ovalbumin	Thiamine-Gantrez® AN NPs	[15]
Tomato lectin (TL)	–	TL-polystyrene microspheres	[87,88]
	–	TL-polystyrene NPs	[63]
	Insulin	TL-lipsomes	[61]
Transferrin (Tf)	Insulin	Tf-Insulin prodrug	[17,89–91]
Vitamin B12 (B_{12})	Cyclosporin A	B12-polysaccharide-based polymeric micelles	[92]
	Erythropoietin (EPO)	B_{12}-EPO bioconjugates	[93]
	Granulocyte-colony stimulating factor (G-CSF)	B_{12}-G-CSF bioconjugates	[93]
	Human PYY (hPYY)	B_{12}-hPYY bioconjugates	[29]
	Insulin	B_{12}-Gel-Core-SLN	[94]
	Insulin	B_{12}-chitosan-calcium phosphate NPs	[95]
	Insulin	B_{12}-dextran NPs	[16,26]
	Insulin	B_{12}-insulin bioconjugates	[96]
	Luteinizing-hormone-releasing hormone (LHRH)	B_{12}-LHRH bioconjugates	[30]
	Ovalbumin	B_{12}-Gantrez® AN NPs	[97]
	–	B_{12}-polystyrene NPs	[27]
Wheat germ agglutinin (WGA)	Birch pollen allergens	WGA-PLGA microspheres	[98]
	BSA	WGA-PLGA nanospheres	[99]
	BSA	WGA-BSA prodrug	[56]
	Bufalin	WGA-lipid NPs	[100,101]
	[^{3}H]DPPC	WGA-liposomes	[102]
	Insulin	WGA-alginate microparticles	[53]
	Insulin	WGA-SLNs	[103]
	Oridonin	WGA-lipid-polymer hybrid NPs	[19]
	Paclitaxel (PTX)	WGA-SLN	[18]
	Thymopentin	WGA-PLGA NPs	[57,58]
	–	WGA-biotin-avidin-biotin-PEG-PCL/PLA NPs	[104]
	–	WGA-PLAG microspheres	[105]
	–	WGA-Polysicences NPs	[60]
APN antibody	Protein G	APN antibody-β-glucan microparticles	[20]

Fc). InsNP-Fc demonstrated a hypoglycemic effect *in vivo* at a dose that was less than that of other insulin delivery systems. The efficacy of FcRn targeting was indicated in FcRn knockout mice [46]. Similarly, FcRn targeting has also been used to enhance the oral delivery of SP141, a murine double minute 2 (MDM2) inhibitor [14]. The Fc portion of IgG was used in this study to modify the surface of maleimidyl-poly(ethylene glycol)-co-poly(ε-caprolactone) (Mal-PEG-PCL) nanoparticles. FcRn-targeted nanoparticles encapsulating SP141 (SP141FcNP) exhibited improved *in vitro* permeability across Caco-2 cell monolayers and were also able to enhance antitumor efficacy *in vivo* in preclinical models of human breast cancer. During the *in vivo* real-time biodistribution study, after oral administration of fluorescent NPs (DiD-NP and DiD-FcNP) and free DiD solution, the DiD-FcNP group demonstrated a significant fluorescent signal in the tumors 2 h postadministration that was maintained up to 48 h postadministration (Fig. 2A). *Ex vivo* tumor accumulation studies exhibited the strongest fluorescence intensity in tumor tissues in the fluorescent FcNP treatment group (Fig. 2B). Therefore, these results demonstrated the ability of Fc-conjugated nanosystems to target and enhance the oral therapeutic efficacy of anticancer drugs for breast cancer treatment [14].

Similarly, formulation scientists have explored the possibility of using the second ligand of the FcRn receptor, albumin, to develop oral delivery systems for drugs with poor bioavailability. Owing to its targeting ability and influence on the pharmacokinetic parameters of encapsulated drug molecules, albumin has been studied extensively as a drug delivery platform. Furthermore, albumin is nonimmunogenic, abundantly available and highly stable with inherent binding capacity [47]. Santo's group demonstrated the ability of albumin-decorated NPs to significantly improve insulin permeability *in vitro* across intestinal cell monolayers. However, these albumin-modified systems have not yet been evaluated *in vivo* [44,48]. Despite numerous advantages, albumin-based oral delivery systems have not been exploited to their full

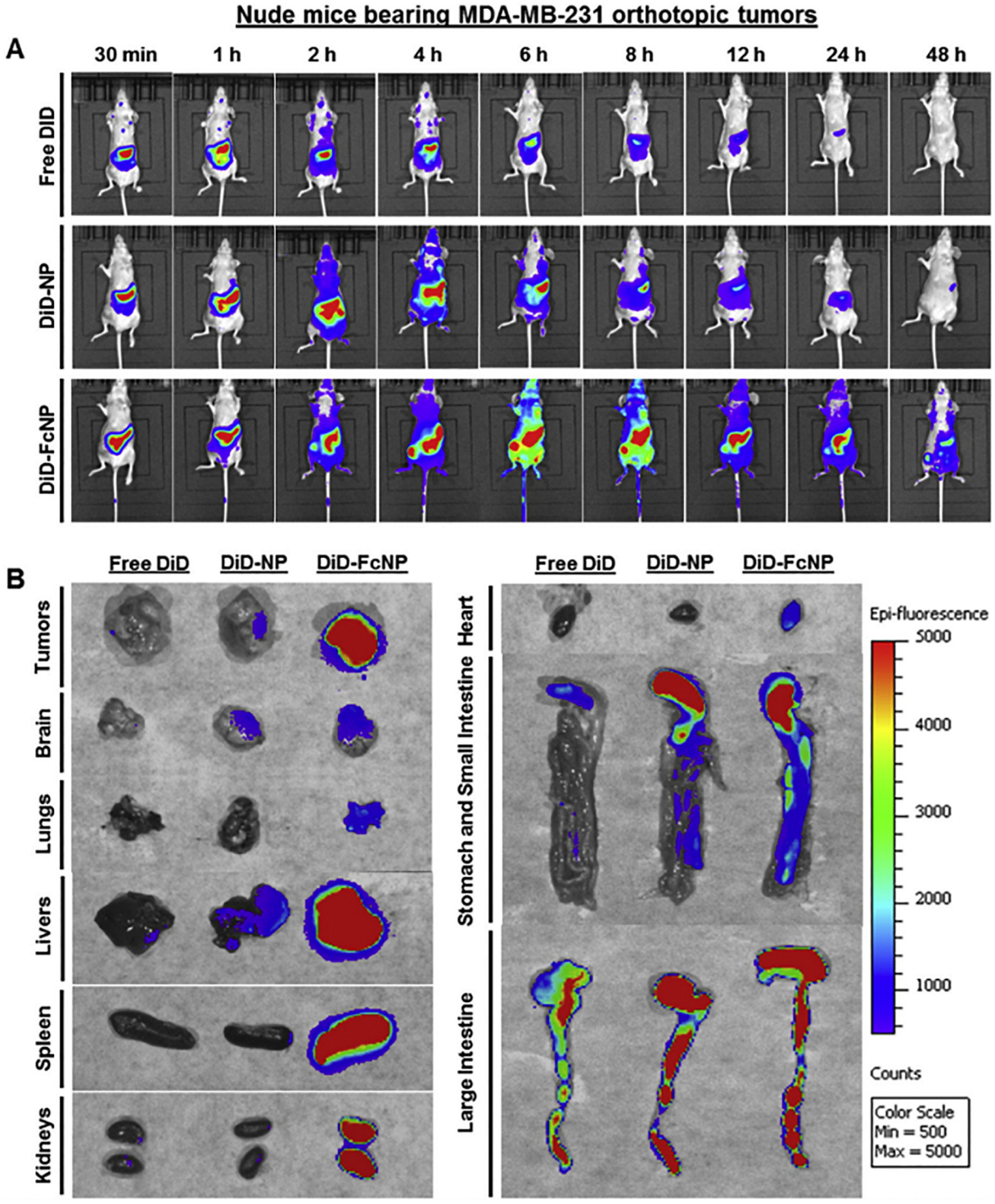

Fig. 2. *In vivo real*-time tumor uptake and *ex vivo* tissue biodistribution of orally administered fluorescent NPs (DiD-NP and DiD-FcNP). (A) *In vivo* time-dependent whole body images of nude mice bearing MDA-MB-231 breast cancer cell orthotopic tumors at a dose of 0.5 mg/kg by oral gavage of free DiD and DiD-labeled NPs with or without ligand. (B) *Ex vivo* images of orthotopic tumors and various tissues from tumor-bearing nude mice that were sacrificed 6 h after oral gavage. DiD-NP and DiD-FcNP have an equivalent dose of DiD in all experiments (n = 3 animals per group). Reproduced, with permission, from ref. [14].

potential. Albumin can not only bind to the FcRn receptor but it can also interact with other cell surface receptors, such as gp18, cubilin and megalin [47]. Therefore, the structure of albumin must be maintained during formulation to avoid a loss of affinity for FcRn and to avoid any unnecessary recognition by other receptors (such as gp18).

FcRn receptors have the ability to bind both IgG and albumin simultaneously without competition [49]. This unique feature of the FcRn receptor can also be exploited to develop a novel dual-targeted system with both ligands (IgG and albumin) to further enhance oral bioavailability.

2.1.3. Lectins

Lectins are naturally present proteins that can be derived from plant or bacterial sources. Lectins have a strong binding affinity for the

carbohydrates attached to proteins and lipids. The potential of using lectin-targeted systems arose from the abundance of glycosylated proteins and lipids found on cell membranes [50]. Naisbett and Woodley first demonstrated that tomato lectins (TLs) strongly bound to rat intestinal mucosa *in vitro* by targeting major glycoproteins that are present on the intestinal brush border [51]. Several studies have shown that lectins present a high affinity for carbohydrate residues on the glycocalyx of intestinal cells and the mucus layer.

Wheat germ agglutinin (WGA) is used to target the intestine as it specifically binds to N-acetyl-D-glucosamine and sialic acid residues present at the intestinal cell surface, resulting in adhesion [52]. WGA has been shown to have the highest binding constant to human intestinal cells and human colonocytes [53]. In addition to binding to the cell surface, WGA can also be endocytosed by enterocytes via receptor-mediated endocytosis [54]. These features of WGA make it an attractive targeting ligand for the intestine. Furthermore, WGA has no oral toxicity and is resistant to the proteolytic enzymes present in the GIT [55]. Therefore, the use of WGA grafting for the oral delivery of therapeutics has garnered considerable attention from formulation scientists. Gabor et al. demonstrated that bovine serum albumin (BSA)-WGA conjugates have significantly higher binding to the Caco-2 cell monolayer; 19-fold higher than BSA alone. The study confirmed the importance of lectin conjugation for bioadhesion [56]. WGA present on the surface of nanocarriers significantly augments the interactions with mucus and the intestinal epithelial surface, thus greatly increasing intestinal permeability. Yin et al. demonstrated that WGA modification of PLGA NPs resulted in a significant increase in systemic uptake of thymopentin after oral administration [57]. The presence of WGA on the surface of the nanoparticles led to strong bioadhesion and augmented intestinal uptake [57,58]. These studies emphasized that the endocytosis of WGA-NPs mainly contributed to enterocyte targeting rather than M cell targeting. In another study, WGA-decorated nanocages firmly adhered to the epithelial surface and subsequently entered into the lamina propria via goblet cells [59]. N-Acetyl-D-glucosamine binding TL has also demonstrated a high affinity for enterocytes [60,61]. Unlike many other lectins, TL presents the advantage of being relatively nontoxic [62]. TL-modified NPs have demonstrated bioadhesion *in vivo,* and significantly higher absorption in the rat gut was observed for TL-decorated NPs compared to unmodified NPs. In the rat GIT, TL-decorated NPs displayed their main interaction with enterocytes [63]. Thus, both WGA and TL have appropriate specificity for enterocytes rather than M cells and/or mucus.

2.1.4. Peptidic ligands

Peptidic ligands that react specifically with surface receptors of enterocytes are being pursued as a potential strategy to improve intestinal drug permeability. Peptides are particularly well suited for targeting because they are small (less than 30 amino acids on average), easy to synthesize and typically nonimmunogenic. Moreover, the presence of multivalent sites of a peptide results in high specificity and avidity for the target [64]. Furthermore, the stability of peptides can be improved by using modified peptides, such N- and C-terminally blocked peptides, D-amino acids, and/or peptidomimetics, which are generally more resistant to proteolysis [65].

Integrins, heterodimeric glycoproteins, are cell receptors that are critical for binding to the extracellular matrix and other cells [66]. Arginine–glycine–aspartic acid (RGD) is one of the most commonly used ligands to target integrin receptors (e.g., the $\alpha_v\beta_3$ receptor) [65]. $\alpha_v\beta_3$ is a member of the integrin family, which is a transmembrane glycoprotein expressed on Caco-2 cells [67]. The FQSIYPpIK (FQS) peptide, which does not belong to the RGD family, was identified as a ligand with high affinity for the $\alpha_v\beta_3$ receptor [68]. Liu et al. developed FQS-modified trimethyl chitosan chloride NPs for oral insulin delivery [21,69]. FQS-modified NPs exhibited significantly accelerated intracellular uptake and transport *in vitro* due to active ligand–receptor mediation [21,69]. FQS-modified NPs demonstrated 25-fold and 1.42-fold higher blood glucose lowering effects than free insulin and unmodified NPs, respectively, in diabetic rats [21]. This newly discovered peptidic ligand has also recently been exploited in tumor-targeting systems by the same group [70,71].

The transferrin receptor (TfR), an iron-transporting glycoprotein, can be found substantially in the small intestine epithelium [72]. HAIYPRH (7pep) is a peptidic ligand that exhibits high affinity for the TfR. Du et al. developed 7pep-modified PEG-b-PCL micelles loaded with coumarin 6 (7pep-M-C6). 7pep-M-C6 exhibited higher intracellular uptake compared with the unmodified nanocarriers in Caco-2 cells and increased the *in vivo* intestinal distribution [22]. Although 7pep-modified micelles have shown efficient transcytosis across the Caco-2 cell monolayer, it should be noted that TfRs are found mainly on the basolateral side of differentiated enterocytes, presenting a major hurdle for targeting [73].

The number of peptides used to increase transport across the gastrointestinal tract is enormous. In the present review, we only highlighted the most remarkable examples regarding their use as targets via drug delivery systems. The review by Sánchez-Navarro et al. [74] provides detailed information about peptides in oral drug delivery, as it is devoted specifically to the use of peptides for increased gastrointestinal absorption.

2.2. Goblet cell targeting

Goblet cells represent ~16 % of the total cells in the small intestine and are responsible for producing, storing and continuously releasing mucins, which is the main component of the mucus layer. They possess a protective barrier function, thereby strongly limiting the absorption of therapeutics [106]. Despite the abundance of this cell line, goblet cells are seldom used as a target. The limited targeting of these cell lines can also be attributed to the presence of very few proven targeting ligands. Until now, the peptide CSKSSDYQC (CSK) has been the only proven potent ligand to target goblet cells to increase the oral delivery of therapeutics [107–109]. Kang and coworkers were the first to identify CSK as a high affinity ligand targeting goblet cells [110]. The authors demonstrated the ability of this ligand to increase the transport of M13 bacteriophages across the intestinal epithelium by targeting goblet cells [110]. This study established CSK as a potential ligand for goblet cell targeting for oral formulations. Jin et al. developed CSK-functionalized trimethyl chitosan nanoparticles (CSK-TMC NPs) aimed at the oral delivery of insulin [107]. CSK-TMC enhanced the uptake of insulin in the Caco-2/HT29-MTX cell monolayer model. It was also demonstrated that the nanoparticles were transcytosed via clathrin- and caveolae-mediated endocytosis [107]. Furthermore, the presence of a mucus layer on the cell monolayers only partially hampered uptake. Similar results were also observed in an *ex vivo* ligated ileum loop model, where FITC-labeled CSK-TMC NPs exhibited greater fluorescence intensity in goblet cells [107]. CSK-modified NPs showed 1.5-fold higher relative bioavailability compared to unmodified NPs in diabetic rats [107]. Other studies further demonstrated that different DDSs modified with the CSK peptide increased the oral bioavailability of other biomacromolecules, such as salmon calcitonin and exenatide [108,111–113]. Gemcitabine-loaded TMC conjugates modified with the CSK peptide have been explored to improve the oral treatment of breast cancer [109]. CSK-TMC conjugates improved the cellular uptake of gemcitabine via goblet cell targeting *in vitro* in mucus-producing cells. An *in vivo* efficacy study demonstrated that both drug-loaded CSK-TMC conjugates (60.1 %) and drug-loaded TMC-NPs (54.0 %) increased the oral bioavailability of gemcitabine over the drug solution, and there was no significant difference between them [109].

Considering the positive results encountered in oral drug absorption through goblet cell targeting, it would be interesting to study a larger variety of ligands that target goblet cells. As an example, Nikitas et al. demonstrated that *Listeria monocytogenes* has the ability to bind to E-cadherin, which is expressed on the apical side of mucus-secreting

goblet cells. Thus, the development of E-cadherin-targeting DDSs could be a promising strategy for goblet cell targeting [114].

2.3. M cell targeting

M cells are specialized epithelial cells that are a part of the mucosal immune system. These cells are found primarily in the follicle-associated epithelium (FAE) of Peyer's patches or gut-associated lymphoid tissues (GALTs) [115]. They have the ability to transport particulate matter, including antigens, bacteria and viruses [116]. Therefore, M cells act as an entrance for microorganism invasion and are involved in initiating antigen-specific mucosal immune responses and food tolerance [117]. In addition, they differ from columnar enterocytes due to their underdeveloped microvilli and glycocalyx structures and reduced levels of membrane hydrolase activity. Furthermore, a limited number of goblet cells in the FAE area results in little or no mucus, allowing for efficient uptake of particles via M cells [118]. Unlike DCs, M cells mainly transcytose antigens without passing through lysosomes, thus avoiding degradation [119]. Because of their special abovementioned features, these cells represent a highly promising target for oral drug delivery and oral immunization strategies [120,121].

Although M cells only encompass < 1 % of intestinal cells (this number differs between rodents and humans), extensive research efforts have established M cells as a target for a wide array of orally administered therapeutic agents, such as peptides, proteins, nucleic acids, and vaccines. Various studies have demonstrated that specific M cell targeting could compensate for the limitations of the low number of M cells for oral drug delivery [23,115,122,123]. Conjugating suitable M cell targeting molecules (such as plant lectins, outer membrane bacterial and viral proteins, and monoclonal antibodies) with DDSs has evolved as one of the main approaches to improve the therapeutic efficacy of orally administered agents [124–127]. Some examples of M cell targeting are summarized in Table 2.

2.3.1. Lectins and lectin mimetics

Lectins are one of the most extensively studied ligands to target M cells [128]. Lectins recognize specific carbohydrate residues present in the FAE glycocalyx, which has been exploited to develop M cell targeting systems. *Ulex europaeus agglutinin-1* (UEA-1) is one of the most commonly used lectins for M cell targeting. It is a fucose-specific lectin with an affinity for glycoproteins with a fucα1-2Galβ core located on the luminal side of M cells [129]. Conjugation of UEA-1 to surface liposomes, polymeric nanoparticles and microparticles has led to uptake by M cells after oral administration [130–132]. However, potential proteolytic degradation, cytotoxicity and immunogenic effects limit the applications of UEA-1 as a targeting agent to deliver therapeutics across the GIT. Moreover, UEA-1 specifically targets M cells of mouse origin and not human origin, greatly limiting its application and clinical translation to humans. In addition to the UEA-1 lectin, lectin mimetics have also been studied to target M cells [133]. TThe ability of a UEA-1 lectin mimetic to mediate M cell-specific delivery was tested *in situ* in a mouse gut loop model using dye-loaded polystyrene particles. UEA-1 lectin mimetics possess characteristics such as low molecular weight, stability, ease of synthesis and low cost, making them ideal candidate ligands for M cell targeting [133]. The efficacy of UEA-1 lectin mimetics has only been demonstrated in mice, and their targeting ability in human M cells remains unclear. Tetragalloyl-D-lysine dendrimer (TGDK), another UEA-1 lectin mimetic, was used for targeted delivery of multiantigens, rhesus C-C chemokine receptor 5 (CCR5)-derived cyclopeptide and bovine serum albumin (BSA). I Its oral administration led to a significant increase in the therapeutic response against a rhesus CCR5-derived cyclopeptide [134]. The specific binding affinity of TGDK for human M-like cells widens its application in primates for M cell-targeted mucosal vaccines [134]. *Aleuria aurantia lectin* (AAL) is another fucose-binding lectin that has specificity for fucα1-6/3GlcNAc and targets murine M cells [129]. It includes the beneficial characteristics of UEA-1, i.e., targeting M cells, while eliminating the adverse toxicity associated with UEA-1. Roth-Walter et al. constructed a novel allergen oral delivery system based on PLGA microparticles conjugated with AAL [124]. An increase in vaccine absorption was also observed after oral administration of the microparticles linked to AAL [135,136]. Galectin 9 and sialyl Lewis A antigen are lectins that are specific for the human FAE [23,115]. However, until now, they have not been explored as targeting ligands to decorate DDSs yet.

2.3.2. Pattern recognition receptor-mediated targeting

As mentioned above, M cells are involved in presenting and processing different types of pathogens and antigens. Therefore, the receptors that are involved in these interactions can be exploited as a strategy for M cell targeting [137]. Various pattern recognition receptors (PRRs) can be found expressed on the surface of M cells and other cells present in Peyer's patches. Toll-like receptor-4 or Toll-like receptor-2 (TLR-4 or TLR-2), platelet-activating factor receptor (PAFR), and $\alpha_5\beta_1$ integrin are some of the interesting PRRs that can be used as potential targets. These receptors interact with pathogen-associated molecular patterns, resulting in the translocation of bacteria across the intestinal lumen [115]. TLR-4 and TLR-2 are receptors for lipopolysaccharide (LPS) expressed by gram-negative bacteria [138–140] and for lipoteichoic acids of gram-positive bacteria [141]. TLR-4 and TLR-2 have binding affinity for the phosphorylcholine (PC) moiety of lipooligosaccharide (LOS) molecules present on the bacterial surface [142], and $\alpha_5\beta_1$ integrin has the affinity to bind to fibronectin-binding proteins expressed by many bacteria (such as *Yersinia*) [143]. *Salmonella Enteritidis*-derived flagellin and monophosphoryl lipid A are TLR ligands that have been used to modify nanoparticle surfaces to target M cells [120,144]. *Yersinia* interacts with invasin, and $\alpha_5\beta_1$ integrins are overexpressed on the apical surface of human M cells, resulting in internalization by M cells. As mentioned previously, the RGD sequence (Section 2.1.4) has a high affinity for integrin receptors. Garinot et al. demonstrated that the grafting of RGD to the surface of PEGylated PLGA-based NPs encapsulating ovalbumin significantly improved the transport of NPs across a human M-like cell model *in vitro*. A slight improvement in the IgG immune response was also observed after oral immunization with RGD-modified NPs *in vivo* [145]. Lee et al. described the use of a β-glucan-functionalized glycine-arginine-glycine-aspartic acid-serine (GRGDS) carrier for the oral delivery of PR8 (an inactivated antigen of influenza A). The resulting M cell-targeted β-glucan-GRGDS/PR8 carrier showed the highest amount of immunoglobulin A (IgA) antibody in the blood serum and mucus 21 days after the first oral dose [146]. RGD peptidomimetics (RGDps) have also been used as targeting ligands to functionalize NPs [147]. Significant improvement in the transport of NPs *in vitro* across an M cell-like human model was observed after the RGDp modification of NPs. Furthermore, similar improved therapeutic efficacy was also observed in *in vivo* studies with RGD-NPs compared to unmodified NPs [147].

2.3.3. Glycoprotein 2-mediated targeting

Glycoprotein 2 (GP2) is a highly expressed transcytotic receptor found on the luminal membrane of M cells. It recognizes FimH, which is a major component of the type I pili on the bacterial outer membrane of a subset of gram-negative enteric bacilli. *Escherichia coli* and *Salmonella enterica serovar Typhimurium* (S. Typhimurium) are some examples of $FimH^+$ bacteria [117,148]. Shima et al. constructed a fusion protein, anti-GP2-streptavidin (SA), to deliver antigens. Biotinylated ovalbumin peptide (bOVA) was conjugated to the fusion protein. The specific binding of anti-GP2-SA to M cells was demonstrated by an immunofluorescence study. Significant induction of OVA-specific fecal IgA secretion was observed after oral administration of the fusion protein in mice [149]. This study further presented anti-GP2-SA as an efficient mucosal vaccine by enduring the harsh gastrointestinal environment and efficiently delivering the antigen to M cells [149]. Matsumura et al. found that serotype A1 L-progenitor toxin complexes (L-PTCs) consisted

Table 2
Examples of different ligands used in active M cell targeting for oral drug delivery.

Ligands	Cargo	Drug delivery systems	References
Aleuria aurantia lectin (AAL)	S-91 cell antigens	AAL-albumin microparticles	[135]
	Birch pollen allergens	AAL-PLGA microspheres	[124]
	ID 8 cell lysate	AAL-microparticles	[170]
	Mycobacterium tuberculosis antigens	AAL-microspheres	[136]
	Melanoma vaccine	AAL-microparticles	[135]
	Whole cell lysate (WCL)	AAL-microparticles	[171]
	–	AAL-microparticles	[172]
Arachis hypogaea (PNA, lectin)	Hepatitis B surface antigen (HBsAg)	PNA-PLGA NPs	[173]
Cholera toxin B subunit (CTB)	Hepatitis B surface antigen (HBsAg)	CTB-bilosomes	[174]
CKSTHPLSC (CKS9)	Brachyspira hyodysenteriae (BmpB)	CKS9-chitosan-PLGA microparticles	[161]
	Interleukin 6 (IL-6)	IL-6-CKS9	[162]
	–	CKS9-chitosan NPs	[160]
Clostridium perfringens enterotoxin (CPE 30)	Coxsackievirus B3 predominant antigen VP1 (pVP1)	CPE30-chitisan-pVP1 NPs	[154]
	Recombinant influenza hemagglutinin (HA)	CPE 30-PLGA sub-micron particles	[126]
	Recombinant influenza hemagglutinin (HA)	CPE30-HA	[153]
Col	Envelope domain III (EDIII)	EDIII-Col complex	[158]
	Core neutralizing epitope (COE)	pPG-COE-Col-DCpep/L393 (*Lactobacillus casei* 393)	[175]
CTGKSC	–	CTGKSC-PEG-PCL/PLGA NPs	[159]
GRGDS	PR8 antigen	β-Glucan-GRGDS NPs	[146]
Glycoprotein 2 (GP2) Ligands	Biotinylated ovalbumin peptide (bOVA)	Anti-GP2-streptavidin-bOVA	[149]
LDV peptidomimetic (LDVp)	Ovalbumin (OVA)	LDVp-PEG-PCL/PLGA NPs	[147]
LRVG	–	LRVG-PEG-PCL/PLGA NPs	[159]
Neuraminidase (NA)	Allergen	NA-PLGA microparticles	[176]
mAb NKM 16-2-4	tetanus toxoid (TT)/botulinum toxoid (BT)	mAb NKM 16-2-4-TT/BT	[163]
Outer membrane protein H (OmpH)	Envelope domain III (EDIII)	EDIII-OmpH complex	[156]
RGD peptide	Antigen heat shock protein 65–6 × P277 (H6P)	RGD- and mannose-modified chitosan (RMCS) NPs	[177]
	Doxorubicin	RGD-conjugated triglyceride-based nanocarrier	[178]
	Ovalbumin (OVA)	RGD-PEG-PCL/PLGA NPs	[145]
	–	RGD- polystyrene particles	[179]
RGD peptidomimetic (RGDp)	Ovalbumin (OVA)	RGDp-PEG-PCL/PLGA NPs	[147]
Salmonella enteritidis extract (SE)	Fluorescein isothiocyanate (FITC)	SE-Gantrez® AN NPs	[180]
Salmonella Enteritidis derived flagellin	Ovalbumin (OVA)	Flagellin-Gantrez® AN NPs	[144]
Secretory IgA (SIgA)	p24HIV antigen	p24-SIgA complexes	[165]
Tetragalloyl-D-lysine dendrimer (TGDK)	rhesus CCR5-derived cyclopeptide/BSA	TGDK-cyclopeptide	[134]
Ulex europaeus agglutinin-1 (UEA-1)	Bovine serum albumin, (BSA)	UEA-1-chiitosan NPs	[130]
	BSA	UEA-1- polystyrene microspheres	[181]
	Dextran rhodamine	UEA-1-polymerised liposome	[132]
	HIV peptides	UEA-1-PLG microparticle	[131]
	H. pylori or C. jejuni	UEA-1-agglutinated H. pylori or C. jejuni	[182]
	Ovalbumin (OVA)	UEA-1-PLGA-lipid NPs	[183]
	Hepatitis B surface antigen (HBsAg)	UEA-1-liposomes	[184]
	Hepatitis B surface antigen (HBsAg)	UEA-1-PLGA NPs	[185]
	–	UEA-1-microspheres	[186]
UEA-1 mimetics	–	UEA-1 mimetics- polystyrene particles	[133]
Wheat germ agglutinin (WGA)	Thymopentin	WGA-PLGA NPs	[57]

of botulinum neurotoxin, nontoxic nonhemagglutinin and hemagglutinin (HA). HA interacts and bids to GP2, thus resulting in the opening of the intestinal barrier (Fig. 3) [150]. Fig. 3A compares the interaction between HA and different binding molecules expressed on M cells that could be possible receptors for toxin complexes. HA strongly binds to both mouse and human GP2 (mGP2 and hGP2). Furthermore, significant colocalization of L-PTCs with GP2 was observed in M cells in intestinal loop assays (Fig. 3B). The involvement of GP2 receptors was proven to be significantly less susceptible in GP2-deficient ($Gp2^{-/-}$) mice than in wild-type ($Gp2^{+/+}$) mice after oral administration of L-PTCs [150]. In addition to exploiting new GP2 ligands, researchers have observed that the number of functional $GP2^{+}$ M cells can be significantly increased by systemic administration of a receptor activator of nuclear factor (NF)-κB ligand [151]. This increase in functional $GP2^{+}$ M cells could then induce mucosal and humoral immune responses upon oral delivery of the antigen via M cell-targeted delivery systems [151].

2.3.4. Claudin 4-mediated targeting

Claudin 4, a transmembrane protein, is located in the tight junctions between intestinal cells as well as on the surface of M cells (human and murine origin). In M cells, claudin 4 acts as a receptor for *Clostridium perfringens* enterotoxin (CPE) by directly binding to its C-terminal 30 amino acids (CPE30) [126,152,153]. Ye et al. designed a CPE30 peptide-modified chitosan-based delivery system (CPE30-CS-pVP1) for M cell targeting. The CPE30-CS-pVP1 vaccine demonstrated *in vivo* oral efficacy after four doses by significantly increasing specific fecal SIgA levels and augmenting the mucosal T cell immune response [154]. Exploiting tight junction proteins to deliver bioactive compounds to the FAE through M cells has also been demonstrated in other studies [126,152]. Ling et al. demonstrated that conjugating CPE30 to the C terminus of influenza HA does not significantly change its binding ability to claudin 4 [152]. Thejani et al. produced submicron size PLGA particles to incorporate this recombinant protein (HA-CPE) to protect against harsh gastrointestinal conditions while largely retaining its targeting function. In addition, the targeted systems showed a considerable increase in the uptake of HA-CPE in Peyer's patches *in vivo* after oral administration [126].

2.3.5. Complement C5a receptor-mediated targeting

The complement C5a receptor (C5aR) can be found on the apical surface of human M-like cells, and these receptors play a major role during antigen uptake [155]. The outer membrane protein H (OmpH) of *Yersinia enterocolitica* is one of the identified ligands of C5aR. Kim and coworkers used this ligand to modify envelope domain III (EDIII) (EDIII-OmpH), the pathogenic antigen of the dengue virus, to develop

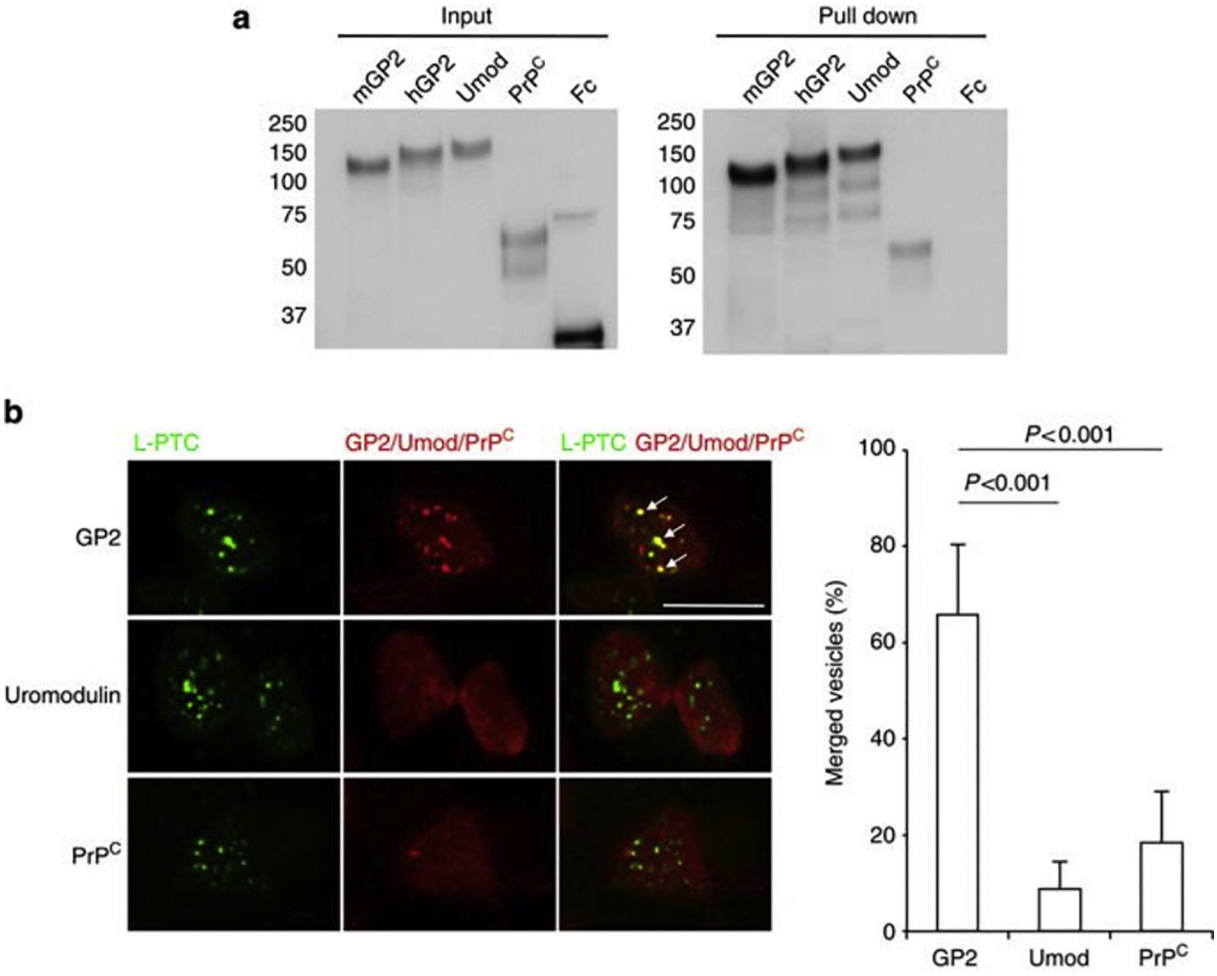

Fig. 3. GP2 serves as a primary endocytotic receptor for the uptake of L-PTC in M cells. (a) HA pull-down assay. Recombinant Fc proteins, including mouse GP2-Fc (mGP2-Fc), human GP2-Fc (hGP2-Fc), mouse uromodulin-Fc (Umod-Fc), and mouse PrP^C-Fc, were incubated with Strep-Tactin Superflow agarose prebound to fully assembled HA (HA1/HA2/HA3). The HA-bound proteins were analyzed by immunoblotting using horseradish peroxidase (HRP)-labeled anti-human IgG antibody. (b) Colocalization of fluorescent L-PTC (green) and GP2, uromodulin and PrP^C (red) in M cells in *in situ* ligated intestinal loop studies. L-PTC predominantly colocalized with GP2 in M cells (arrows). Scale bar, 10 mm. Reproduced, with permission, from ref. [150]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

M cell-targeted antigen delivery systems. An enhancement in the EDIII-specific mucosal and systemic immune responses without systemic tolerance against EDIII was observed after oral administration of EDIII-OmpH [156], demonstrating that it is an effective oral mucosal vaccine against DENV infection. Co1 is another C5aR-targeting peptidic ligand [157], that was used by the same group to successfully deliver the EDIII antigen into Peyer's patches and demonstrate virus-neutralizing activity after oral administration [158].

2.3.6. Peptidic ligands

A number of peptide sequences have been discovered that have been confirmed to interact with unknown ligands present on the M cell surface. For instance, CTGKSC and LRVG are two peptides that have shown enhanced phage transport across M-like cells. LRVG and CTGKSC peptide-decorated PEGylated PLGA-based nanoparticles exhibited an eight- and four-fold increase in their transport in *in vitro* coculture cell lines (M cell-like model [116]), respectively, when compared to nonmodified NPs [159]. Another M cell-homing peptide (CKSTHPLSC (CKS9)) was reported by Cho et al., and was selected for grafting onto chitosan NPs (CKS9-CNs). CKS9-CNs demonstrated increased *in vitro* transport and accumulation in PP regions in an *ex vivo* closed ileal loop assay [160]. Moreover, CKS9 water-soluble chitosan (WSC)-PLGA microparticles were developed encapsulating membrane protein B of *Brachyspira hyodysenteriae* (BmpB) (BmpB-CKS9-WSC-PLGA MPs). After oral administration, the microparticles were able to elevate the systemic IgG antibody response, which was accredited to M cell targeting [161]. Li et al. reported a novel mucosal adjuvant, IL-6-CKS9, which is a recombinant cytokine. IL-6-CKS9 is formed by chemically crosslinking CKS9 with murine interleukin 6 (IL-6). In this study, a recombinant lactic acid bacteria secreting IL-6-CKS9 was orally administered with a CKS9-conjugated antigen protein to BALB/c mice for mucosal immunization. After oral administration, an increase in oral bioavailability was observed along with an increase in the induction of immune responses (Th1- and Th2-type) [162]. Thus, such hybrid delivery systems present an interesting approach to achieve efficient oral vaccine delivery.

2.3.7. Antibodies

The use of M cell-specific antibodies has also been utilized in developing targeted systems. In addition to anti-GP2-SA (as previously described), NKM 16-2-4 mAb is another monoclonal antibody against M cells. Nochi et al. demonstrated the use of NKM 16-2-4 mAb for M cell-targeted oral mucosal vaccine delivery. When NKM 16-2-4 was conjugated to tetanus toxoid (TT) or botulinum toxoid (BT) and administered together with an adjuvant, the resulting oral vaccination resulted in the induction of high-level, antigen-specific serum and mucosal

immunoglobulin responses [163]. Another interesting study was performed by Rochereau et al., who demonstrated the M cell targeting ability of secretory IgA (SIgA) via the dectin-1 receptor. The interaction resulted in the transportation of SIgA in the GALT [164]. This specific targeting approach was explored for the oral delivery of p24HIV antigen by forming a complex with SIgA (p24-SIgA). Oral administration of this complex resulted in the induction of immunity, humoral and cellular, against p24 at both the systemic and mucosal levels [165].

2.3.8. Other ligands

In addition to the abovementioned commonly used ligands for M cell targeting, several studies have found other novel ligands that present specific binding affinity to M cells. A novel ligand was recognized from the outer capsid protein of retrovirus, protein σ1 (pσ1). pσ1 has the ability to specifically interact with the α-(2,3)-linked sialic acid-containing glycoconjugates present on the luminal side of M cells [166–168]. However, no further studies have exploited it as a ligand to orally deliver antigens and/or drugs into Peyer's patches.

Caveolin-1 is a scaffolding protein that is a main part of caveolae expressed on M cells. A possible role of caveolin-1 in enhancing the susceptibility of M cells towards *Salmonella* infection has been identified *in vitro* in cocultured intestinal cell lines (Caco-2 and Raji B cell cocultures) [169]. Thus, caveolin-1 is involved in the M cell-mediated entrance of microbial pathogens and has been established as a promising new approach for achieving mucosal immunity via M cell targeting.

2.4. Dendritic cell targeting

Dendritic cells (DCs), also known as antigen-presenting cells (APCs), play a key role in protective immunity against pathogens by synchronizing innate and adaptive immunity. Furthermore, they are also responsible for maintaining the tolerance of the commensal flora against bacteria, food antigens and self-antigens [187]. These cells have the ability to accurately recognize different signals in their surrounding area and respond to these signals to induce appropriate immune responses. Intestinal DCs are a small subset of DCs that include a large network in the intestinal immune system. These cells are distributed throughout the gut, including the lamina propria of the intestine, Peyer's patches and mesenteric lymph nodes [188]. As shown in Fig. 4A, DCs underlying M cells can extend their dendrites between the intestinal cells to recognize the antigens that are present in the intestinal lumen. The highly efficient interactions of DC surface receptors and administered vaccines has opened the possibility of using DCs to enhance the immunogenicity of oral vaccines [177,189]. A number of receptors present on the surface of DCs (Fig. 4B) are involved in the interaction and transport of foreign molecules and the induction of immune responses. TLRs, FcRns, C-type lectin receptors (CLRs), mannose, integrins and scavenger receptors are some of the receptors that are present on the surface of DCs [190]. Mannose receptors are well-known receptors expressed on DCs that offer the potential to target vaccines to DCs. However, depending on the specific receptor being targeted, the outcome of the resulting immune response may vary. Moreover, the expression of these receptors can vary between their location and DC subset, as each subset has unique patterns for antigen recognition, capture, processing and presentation. For example, TLRs are distributed on the cell membrane surface [191], enabling them to deliver a strong activating signal to DCs, which forms the basis for their potent adjuvant properties of TLRs. CLRs are key receptors that stimulate intracellular signaling cascades. They facilitate receptor-mediated endocytosis by binding to carbohydrate ligands [192]. FcγR is a receptor that binds to the Fc portion of IgG antibodies. These receptors are involved in several immunological processes, such as antigen presentation, DC maturation and the activation of natural effector cells [193].

Owing to the interesting properties of DCs, targeting of these cells has been widely studied for systemically delivered vaccines. Targeting of DCs is achieved by conjugating the appropriate antigen to a specific antibody or ligand with immunostimulatory adjuvants. This method is not suitable for the oral administration of vaccines since antigens are susceptible to degradation in harsh gastrointestinal milieu. Thus, developing delivery systems for vaccines is crucial; as such, delivery systems can improve immune responses by enhancing the endocytosis of antigen-loaded carriers. However, there are only a handful of studies (as mentioned Table 3) that have developed proper systems to specifically target intestinal DC subsets.

Recently, antibodies or ligands have been used to decorate the surface of oral vaccine delivery systems aimed towards DC targeting (Table 3). Beart et al. reported DC-targeting functional β-glucan microparticles (GPs) decorated with antiaminopeptidase N (APN)-specific antibodies for the oral delivery of the antigen FedF (the tipadhesin of F18 fimbriae) [194]. APN is an epithelial receptor present on different

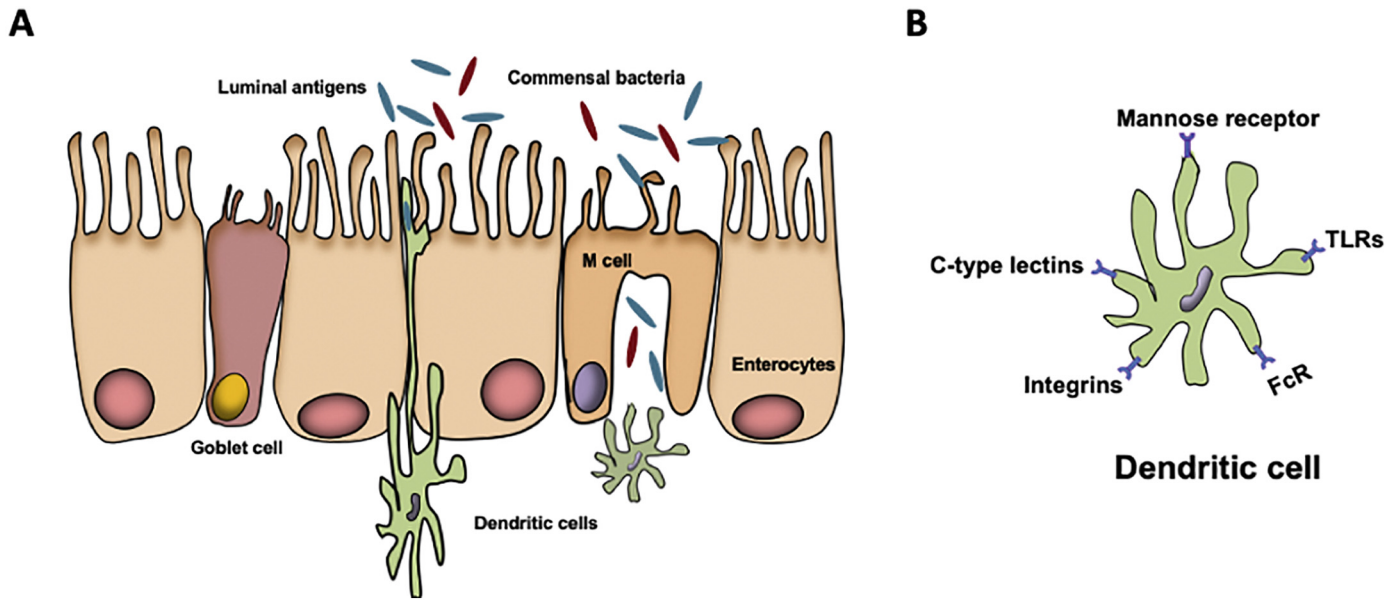

Fig. 4. (A) Schematic representation of the uptake mechanisms of luminal antigens by DCs. (B) Examples of well-known receptors on DCs, which may be potential targeting receptors for oral delivery.

Table 3
Examples of preclinical studies of active dendritic cell-targeting strategies in oral drug delivery.

Ligands	Cargo	Drug delivery systems	References
Antibody (mAB) IMM013	Antigen FedF	Anti-aminopeptidase N(APN) FedF-β-glucan microparticles (GPs)	[194]
DCpep	Core neutralizing epitope (COE)	pPG-COE-Col-DCpep/L393 (*Lactobacillus casei* 393)	[175]
Mannose	Antigen heat shock protein 65-6 × P277 (H6P)	RGD- and mannose-modified chitosan (RMCS) NPs	[177]
	Ovalbumin (OVA)	Mannosylated PLGA NPs	[147]
	Ovalbumin (OVA)	Mannosylated Gantrez® AN NPs	[144]
	Rhodamine B isothiocyanate (RBITC)	Mannosylated Gantrez® AN NPs	[201]

Table 4
Summary of gut hormones secreted by enteroendocrine L cells and K cells and their physiological functions.

Hormones	Site of secretion	Physiological functions	Physiopathological situations
GIP	K cells; proximal small intestine	Induces insulin secretion; inhibits GI motility, secretion of gastric acid and apoptosis of the pancreatic beta cells; reduces lipoprotein lipase activity in adipose tissue.	T2DM; Alzheimer's disease
GLP-1	L cells; duodenum, jejunum, distal ileum, colon	Incretin effect on insulin secretion; promotes pancreatic β cells proliferation and neogenesis; delays gastric emptying; postprandial satiety; inhibits energy intake	Obesity; T2DM; Insulin resistance
GLP-2	L cells; distal small intestine, colon	Stimulate cell proliferation and inhibits apoptosis; promotes mucosal repair; reduces inflammatory responses to bowel injury; maintains the mucosal permeability barrier	Inflammatory bowel diseases (Crohn's disease); short bowel syndrome; osteoporosis
PYY	L cells; ileum, colon	Slows gastric emptying and inhibits intestinal motility; inhibits gastric acid secretion and pancreatic exocrine function; suppresses appetite; stimulates mucosal enterocytes proliferation	Obesity
Oxyntomodulin	L cells; colon	Inhibits gastric acid; reduces gastric mobility; suppresses appetites	Obesity

cells, such as intestinal enterocytes and DCs. *In vitro* studies in DCs demonstrated efficient internalization and DC maturation induction. Such promising effects of the modified GPs were attributed to the combined effects of APN targeting, FcγR employment and β-glucan recognition [194]. In addition, this study further evaluated intestinal DC targeting *in vivo* in piglets. After oral administration, APN-targeted FedF-GPs prompted a significantly higher systemic antibody response against FedF compared to that of control microparticles [194]. Thus, the APN-targeted FedF-GPs were internalized by intestinal cells and induced DC maturation.

Mannose has been used as a ligand for the enhanced targeting of vaccines to intestinal DCs. The mannose receptor (MR/CD206) is highly expressed on DCs [195], and has eight C-type carbohydrate recognition domains (CRDs) per polypeptide [196]. The receptor recognizes mannose-presenting antigens and mediates their endocytosis, processing and presentation [197]. Chen et al. employed mannose as a ligand to develop DC targeting chitosan (CS) NPs, encapsulating the antigen heat shock protein 65-6 × P277 (H6P) [177]. The mannose-decorated nanoparticles demonstrated higher uptake in the Peyer's patches of DCs. Furthermore, oral vaccination with mannose-decorated antigen-containing nanoparticles induced antigen-specific T cell tolerance and completely inhibited the induction of diabetes in NOD mice [177].

Lactobacillus strains have also been used for the development of mucosal vaccines aimed at efficient oral antigen delivery [198,199]. However, the number of antigens reaching the targeted immune site via these recombinant systems is very limited. A DC-targeting peptide-modified oral recombinant *Lactobacillus casei* 393 vaccine was reported to orally deliver the core neutralizing epitope against porcine epidemic diarrhea virus (PEDV) [175]. The DC-targeted recombinant *Lactobacillus* strain was able to induce anti-PEDV mucosal, humoral, and cellular immune responses. Effective induction of the immune response was achieved due to the resistance of *Lactobacillus casei* 393 to the harsh gastrointestinal milieu and its ability to form colonies in the GIT of swine and mice [200]. The promising results from this study unveiled new opportunities for PEDV vaccine development.

Although antigen-loaded DDSs modified towards DC targeting have significantly increased the endocytosis of antigens by specifically interacting with DC subsets, most studies of intestinal DCs are targeted to only one DC subset. Therefore, one feasible strategy would be combinatorial targeting of multiple DC subsets at the same time. A deep understanding of the uptake mechanism after targeting multiple intestinal DC subsets is required to elucidate whether this method could be more efficient than current oral vaccines.

2.5. Enteroendocrine cell targeting

Enteroendocrine cells (EECs) are found interspersed throughout the whole GIT, from the stomach to the rectum, and account for approximately 1 % of the total intestinal cells [202]. EECs constitute the largest endocrine system in the human body; more than twenty different hormones are secreted from 12 types of intestinal EECs. These enteroendocrine hormones moderate their effects via auto-, neuro-, and paracrine mechanisms [203,204]. Gut hormones physiologically regulate multiple biological effects, such as food intake, gastric emptying, intestinal motility, glucose metabolism and intestinal barrier function [23].

L and K cells are the most interesting enteroendocrine cells. They are responsible for secreting different gut hormones that have been proven to have therapeutic efficacy in different prevalent diseases, such as inflammatory bowel disease (IBD) and type 2 diabetes mellitus (T2DM). The gut hormones produced by K and L cells and their physiological functions are summarized in Table 4. GLP-1 and GIP have established roles in gastrointestinal function, glucose homeostasis and satiety. Owing to their insulinotropic effects, GIP and GLP-1 are responsible for more than half of the total insulin secretion after the oral administration of glucose, which is also known as *incretin effect* [205]. These incretin peptides have an extremely short half-life because of their rapid degradation by the enzyme dipeptidyl peptidase (DPP)-4 within a few minutes [206]. Until now, there have been two classes of drugs based on the incretin effect that are available on the market for the treatment of obesity/T2DM, including orally administered DPP-4 inhibitors (e.g., linagliptin, saxagliptin and sitagliptin) and GLP-1 mimetics (e.g., liraglutide, semaglutide and exenatide). Notably, GIP has demonstrated lower therapeutic effectiveness in T2DM treatment than GLP-1. GLP-2 and peptide YY (PYY) are also released from intestinal L cells after the ingestion of nutrients, including carbohydrates and fat.

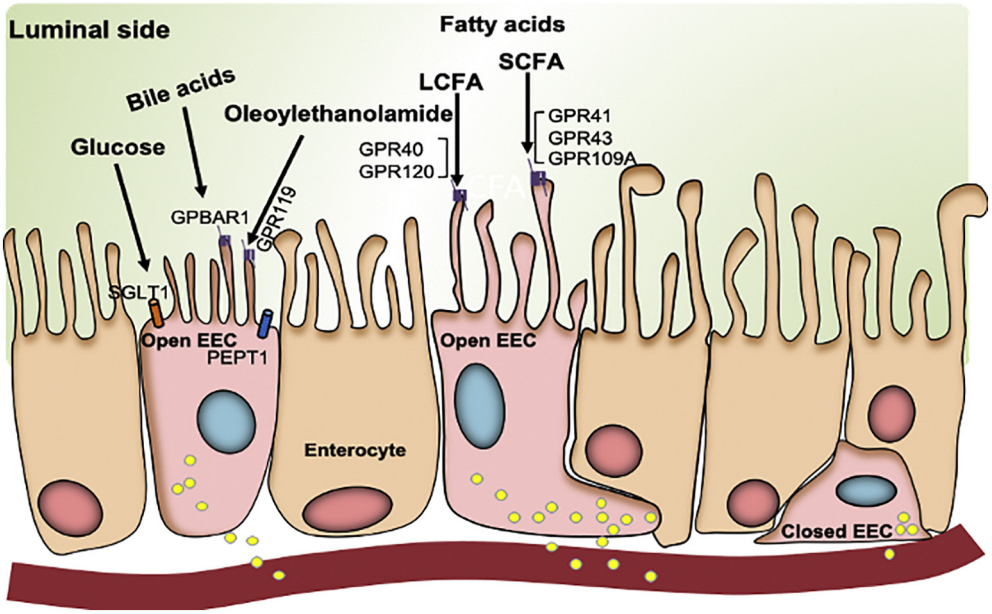

Fig. 5. Graphic overview of the morphology of enteroendocrine cells (EECs) and the well-known receptors on the surface of open EECs. Open-type EECs are covered by microvilli and directly reach the luminal surface, whereas closed-type EECs lack microvilli and are located close to the basal side in the epithelia and do not reach the gut lumen. Open EECs (such as enteroendocrine L cells and K cells) express various nutrient-sensing proteins, including G protein-coupled receptors (GPCRs), such as GPR40, GPR41, GPR43, GPR119 and GPR120, and nutrient transporters, such as SGLT1 and PEPT1. Theses receptors and nutrient transporters are activated by different dietary nutrients in the intestinal lumen (e.g., carbohydrates, proteins, and lipids) and thus modulate the secretion of EEC hormones.

Intestinal GLP-2 is cosecreted along with GLP-1, stimulating cell proliferation and inhibiting apoptosis. PYY reduces food intake and induces food aversion, and in combination with GLP-1, PYY plays a major role in the ileal brake, a physiological mechanism that delays intestinal motility and transit to enhance nutrient (mainly lipid) absorption [202]. Targeting L and/or K cells, therefore, represents an exciting new therapeutic opportunity in some major gastrointestinal diseases.

The apical membrane of enteroendocrine L and K cells expresses a wide variety of receptors and transporters (Fig. 5). These receptors and transporters could be activated by different dietary nutrients (e.g., carbohydrates, proteins, and lipids) and then regulate gut hormone release from EECs. Some G protein-coupled receptors (GPCRs) are lipid-sensing receptors, such as free fatty acid receptors (FFARs) 1-4. FFAR2 (GPR43) and FFAR3 (GPR41) are highly expressed in colonic L cells and activated by short-chain fatty acids (primarily acetate (C2), propionate (C3), and butyrate (C4)), thereby stimulating GLP-1 secretion into blood circulation [207]. FFAR1 (GPR40) and FFAR4 (GPR120) are responsive to medium-chain fatty acids (C7 to C12) and long-chain fatty acids (C12 to C18) [208]. GPR119 is a lipid derivative receptor that is activated by fatty acid amides (e.g., oleoylethanolamide). This receptor is involved in regulating the secretion of GLP-1, GLP-2 and PYY [209]. EECs also respond to luminal stimuli other than nutrients, such as bile acids. The G protein–coupled bile acid receptor GPBAR1 (TGR5) is highly expressed in distal gut L cells [210]. Apart from these GPCR sensors, some nutrient transporters, including the sodium-coupled glucose transporter SGLT1 and peptide transporter PEPT1, also participate in the modulation of gut hormone secretion from EECs.

To our knowledge, to date, only a few studies have illustrated enteroendocrine cell targeting in oral drug delivery, and these studies were all reported by the same research group [211–214]. Rather than grafting one of these certain ligands on the surface of a drug delivery system, Beloqui et al. first introduced the concept that lipid-based nanoparticles can act as endogenous ligands stimulating higher GLP-1 release via lipid-sensing pathways in L cells. The researchers quantified the abilities of different lipid-based and polymeric NPs to trigger GLP-1 secretion *in vitro* in L cells. Among the different tested lipid-based and polymeric NPs, the nanostructured lipid carrier (NLC) was the only nanocarrier that could significantly trigger more secretion of endogenous GLP-1 from murine and human L cells, owing to its interactions with the GPR41 and GPR84 receptors on the surface of L cells [211]. This study describes a potential novel targeting strategy by exploiting the inherent targeting potential of nanocarriers without external ligand modification. Two GLP-1 analogs (exenatide and liraglutide) were encapsulated within these NLCs, which was anticipated to induce endogenous GLP-1 secretion while providing increased systemic absorption of the encapsulated GLP-1 analog. However, NLCs mainly adhered to the mucus layer and did not come in close proximity to L cells and failed to trigger GLP-1 secretion *in vivo* [212]. A recent study from the same group evaluated the ability of lipid nanocapsules (LNCs) of different sizes to trigger GLP-1 secretion from L cells. They concluded that empty LNCs with a size of 200 nm could significantly increase GLP-1 secretion both *in vitro* in murine L cells and *in vivo* in normoglycemic mice after oral administration [213]. This proof-of-concept study was the first to demonstrate that lipid-based nanocarrier could mimic endogenous ligands to improve the stimulation of GLP-1 *in vivo*. To exploit this physiological effect exerted by the nanocarriers, Xu et al. developed a dual-action lipid nanocarrier for the treatment of T2DM via the oral route, which combined two actions: (i) increased endogenous GLP-1 secretion triggered by the nanocarrier alone and (ii) improved systemic concentration of a GLP-1 analog (exenatide) that was encapsulated in this drug carrier [214] (Fig. 6). The developed nanosystem triggered greater GLP-1 release both *in vitro* when coincubated with human and murine L cells and *in vivo* when orally administered to high-fat diet (HFD)-induced T2DM mice. Furthermore, these nanocarriers were able to increase exenatide bioavailability up to 4 % when orally administered to obese/diabetic mice compared to the peptide administered in solution. The combination of the lipid nanocarriers with exenatide (increased endogenous GLP-1 secretion and improved systemic concentrations of the GLP-1 analog) was capable of normalizing the glycemia of obese/diabetic mice after both acute (single dose) and chronic/long-term (daily administration for 1 month) treatment (Fig. 7). Intriguingly, following 5 weeks of daily administration, only the nanocarriers encapsulating exenatide (EXE RM LNCs) were capable of lowering glucose levels to the same level as that of the healthy control group (Fig. 7). In addition, after 1 month of treatment, this new formulation reduced the levels of key inflammatory markers that are strongly associated with insulin resistance in obesity/T2DM (e.g., F4/80). This study strongly proved that targeting enteroendocrine L cells represents an innovative and clinically valuable strategy for T2DM treatment.

Although EEC targeting in oral delivery is still in its infancy, the studies published so far on L cell targeting have demonstrated great potential for the treatment of gastrointestinal disorders. Future research will not only explore other alternative L cell-targeting approaches but

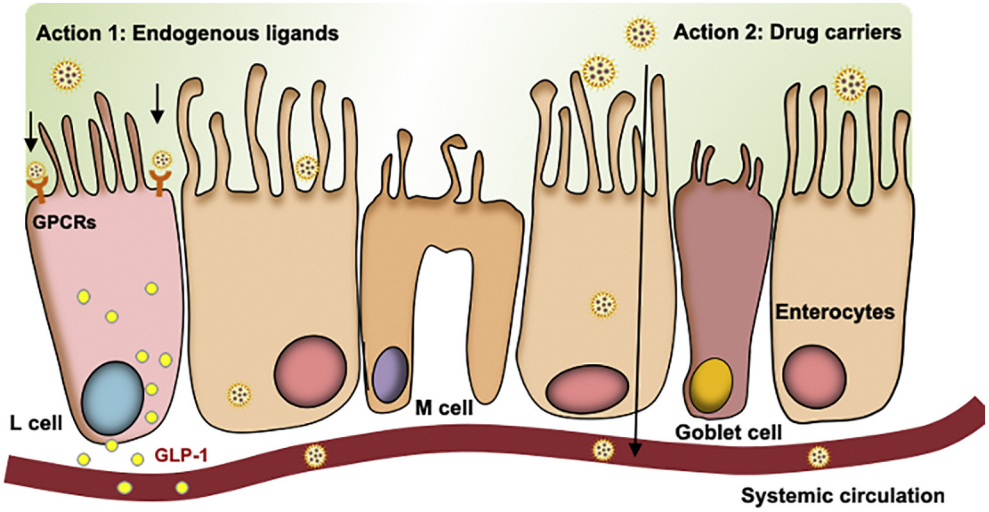

Fig. 6. A graphic representation of the dual-action strategy followed by a lipid-based nanosystem for increased GLP-1 secretion/bioavailability in T2DM treatment. On one hand, lipid-based nanosystems trigger the physiological secretion of GLP-1 by L cells, thus acting as an endogenous ligand of these cells (action 1). On the other hand, these nanocapsules are expected to increase the systemic circulation of the encapsulated GLP-1 analog (action 2). These two actions combined within one nanocarrier represent a promising strategy for T2DM treatment via the oral route.

also develop other EEC-targeting (e.g., K cells) strategies for oral therapy. Moreover, as potential pharmacotherapies in the future, multiple agonists (e.g., a GIP/GLP-1 coagonist, a glucagon/GLP-1 coagonist and GIP/GLP-1/glucagon triagonists) and combination therapy of PYY/GLP-1/oxyntomodulin are being investigated and have shown more effectiveness than GLP-1 alone [215,216]. Thus, a gut hormone agonist-loaded oral drug delivery system with multiple ligands targeting GPCRs and/or nutrient transporters on L/K cells simulating at least two types of functional hormones secreted in the intestine would be a new vista in the EEC-targeting field for a better clinical therapeutic effect.

2.6. Paneth cell targeting

Paneth cells are highly specialized cells that are strongly involved in assisting in the maintenance of the microbiome and can be found at the foot of the crypts of small intestinal villi. Moreover, they are also responsible for establishing the stem cell niche, promoting cell renewal and mucosal morphogenesis [11]. Paneth cells have a turnover of approximately 60 days [217]; thus, they might be a good target compared with enterocytes that only survive several days. The biological injuries of Paneth cells are closely associated with the pathogenesis of different intestinal diseases [11], such as Crohn's disease and enterocolitis [218]. Wang et al. recently identified improved Paneth cell function through activation of the interleukin 22 (IL-22)/phosphorylated Stat3 (p-Stat3) pathway. This study indicated that the IL-22/Stat3 pathway could be a potential target for repairing total parenteral nutrition-related intestinal barrier damage [219]. Furthermore, Paneth cells are also associated with the release of growth factors and antimicrobial peptides, thus playing a vital function in protecting the small intestine [220]. Toll-like receptor 9 (TLR9), expressed on the surface of Paneth cells, recognizes bacterial DNA containing the sequences of unmethylated cytidine–-phosphate–guanosine (CpG) dinucleotides. A study has demonstrated that the oral delivery of oligonucleotides containing a CpG sequence (CpG-ODNs) led to rapid Paneth cell degranulation. Moreover, mice pretreated with CpG-ODNs exhibited increased resistance to oral challenges with virulent *Salmonella typhimurium* [221]. Rumio et al. further investigated the different TLRs expressed on Paneth cells by orally administering TLR agonists. This study demonstrated that in addition to CpG-ODNs (TLR9 agonists), the TLR3 agonist polyinosinic-polycytidylic acid also produced rapid Paneth cell degranulation [222]. TLR9 and TLR3 agonist-induced Paneth cell degranulation provides a pathway towards preventing infection and treating inflammatory bowel diseases. Unfortunately, there is no specific example that has been applied for the treatment of related diseases by using Paneth cell targeting since the mechanisms underlying Paneth cell function remain unknown.

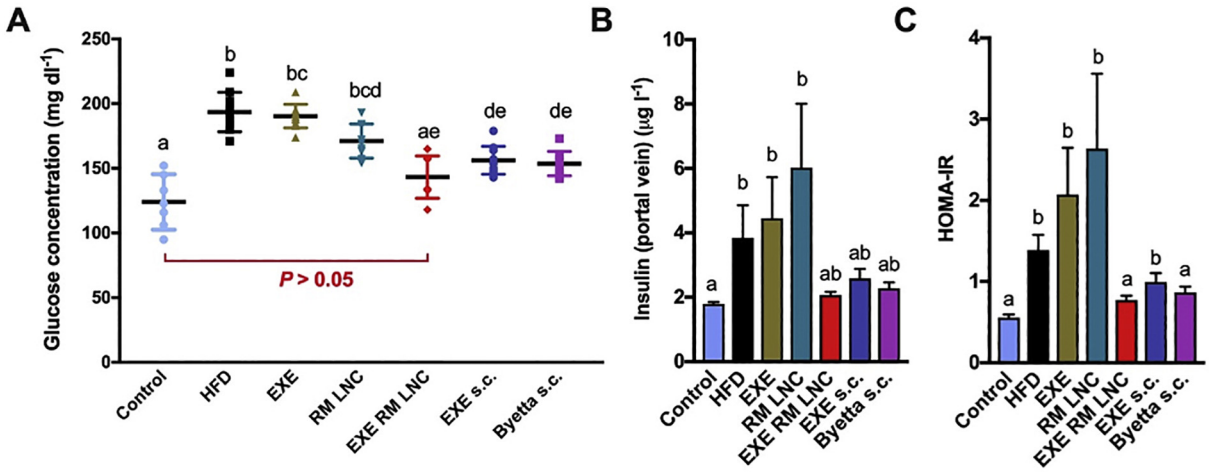

Fig. 7. Effects of EXE RM LNCs on glucose homeostasis and hyperinsulinemia in T2DM mice following chronic treatment (5 weeks). (A) Plasma glucose levels (mg/dL) after 5 weeks of treatment (13 weeks of HFD feeding). (B) Plasma insulin levels collected from the portal vein in sacrificed mice. (C) The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the following equation: [fasting glucose (mg/dL) × fasting insulin (ng/mL)]/405. Reproduced from ref. [214].

3. Transporter targeting

One possibility towards an increased drug delivery system-cell interaction is targeting of the transporters expressed on the intestinal epithelial cell surface. Membrane transporters expressed across the GIT are classified into adenosine triphosphate (ATP)-binding cassette (ABC) transporters and the solute carrier (SLC) transporter superfamily [223]. ABC transporters mainly include efflux transporters (e.g., p-glycoprotein (P-gp) and multidrug resistance-associated protein), which are responsible for limiting the disposition of toxic substances [224]. However, these efflux transporters can have diminishing effects on the transport of therapeutic drug molecules. Furthermore, these efflux transporters are also involved in multidrug resistance. To overcome such efflux, a number of inhibitors of ABC transporters have been identified. Such inhibitors are usually nontoxic compounds that specifically inhibit efflux with high potency without a negative impact on the major drug [225]. Verapamil, cyclosporin A, and trans-flupenthixol, etc., are some of the commonly used P-gp inhibitors [226]. In contrast, SLC transporters are involved in vital physiological activity (e.g., nutrient absorption) and the uptake and transport of several important drug molecules (such as acyclovir, saquinavir and docetaxel) [227]. The majority of SLC transporters (especially influx/uptake transporters) are expressed on intestinal epithelia (on both the apical and basolateral sides), thus making them an attractive targeting site to improve the oral bioavailability of drugs. These transporters mainly include the oligopeptide transporter PEPT1 [228,229], the apical sodium-dependent bile acid transporter (ASBT) [230], the sodium-dependent vitamin transporter SVCT1, the sodium-dependent multivitamin transporter (SMVT), the monocarboxylate transporter MCT1 [231,232], amino acid transporters (LAT1 [233,234] and $ATB^{0,+}$ [235,236]), and the organic cation/carnitine transporter OCTN2. In-depth understanding of the substrate specificity of these transporters allows for the modification of drug moieties (prodrug) or the surface of the nanocarrier to achieve optimal intestinal targeting and an enhanced therapeutic outcome. This part of the review will provide an overview of the targeting strategies selective for specific transporters. Examples are shown in Table 5.

3.1. Oligopeptide transporter PEPT1

PEPT1 is a low-affinity and high-capacity transporter with the unique ability to not easily be saturated by a high concentration of orally administered drugs in the GI tract. Owing to this peculiar function, PEPT1 has captured the attention of the oral drug delivery community. PEPT1 is expressed on the apical membrane of the small intestine (predominantly in the duodenum) and has only limited or no expression in the normal colon [237,238]. PEPT1 is involved in the absorption of nitrogen as well as in the transport of peptide-like therapeutic agents, such as angiotensin-converting enzyme inhibitors, β-lactam antibiotics, and bestatin [239]. Considering its extensive substrate specificity, targeting of the transporter PEPT1 has been developed as a promising approach to increase the oral systematic absorption of poorly permeable drugs [240].

PEPT1-targeting prodrugs have been widely exploited, in which parent drugs are linked to a natural amino acid to become dipeptide analogs (e.g., acyclovir [241], didanosine [242] and L-dopa [243]) or conjugated with a dipeptide to become tripeptide analogs with a higher binding affinity (e.g., acyclovir [244,245], glucosamine [246], lopinavir [247], oleanolic acid [248] and saquinavir [249]). In addition to prodrug strategies, some researchers have used functionalized nanocarriers to load poorly absorbed drugs to target the PEPT1 transporter. Gourdon et al. formulated a PEPT1-targeting nanoparticle by nanoprecipitation for the oral delivery of acyclovir. Valine was used to modify the surface of PLA-PEG-based nanoparticles, which resulted in improved drug permeability in Caco-2 cells by the interaction with intestinal PEPT1 [250]. The same group also encapsulated the peptide oxytocin into different PEPT1-targeting PLA-PEG-based nanoparticles using a number of techniques. Among these PEPT1 transporter-targeted formulations, oxytocin encapsulated by a double emulsion exhibited a drug loading of approximately 4 % (wt/wt) and significantly increased the drug plasma concentration after oral administration compared to that of the free drug in solution [251]. Recently, researchers further demonstrated that PEPT1-targeted micelles and polymeric NPs were promising nanocarriers for enhancing the intestinal absorption of hydrophobic molecules (e.g., docetaxel and curcumin) [252,253]. Of note, various drugs (e.g., anti-inflammatory therapeutics, Lys-Pro-Val, KPV), essentially dipeptides and tripeptides, are mainly mediated via the PEPT1 transporter [254]. PEPT1 is overexpressed in the chronically inflamed colon, although human PEPT1 is not expressed in the normal colonic epithelium [255]. This unique relationship indicates that PEPT1 might be a novel and promising target for IBD treatment. Recently, Zeng et al. developed a fluorescent peptide receptor-targeted probe (dicyanomethylene-4H-pyran-KPV, DCM-KPV). This fluorescent probe led to a significant breakthrough in the recognition and diagnosis of colonic inflammatory regions through direct noninvasive observation. This fluorescent probe also allowed successful differentiation between the chronic, acute ulcerative colitis group and normal groups [256]. This is the first report on a PEPT1-targeted fluorescent probe to differentiate inflammatory activity in ulcerative colitis models *in vivo*. Although dipeptides and tripeptides such as KPV have PEPT1-targeting specificity, their therapeutic efficacy is still very low after oral administration because they hardly accumulate in colitis tissues and are rapidly eliminated by diarrhea. Tailoring nanocarrier-based drug delivery systems with these dipeptide or tripeptide drugs could greatly improve their oral therapeutic efficacy by not only protecting the dipeptides and tripeptides from the harsh GI tract but also allowing further modification on the surface of the nanocarrier (such as decorating ligands that could target specific cells or tissues).

3.2. Apical sodium-dependent bile acid transporter (ASBT)

ASBT, mainly expressed in the apical region of ileal enterocytes, contributes to the enterohepatic recirculation of bile acids. It is expressed more abundantly in the distal ileum than in the proximal ileum [230]. Bile acid-drug conjugates (prodrugs) could effectively improve the intestinal absorption of poorly soluble drugs. One of the most interesting strategies studied so far is the development of acyclovir-bile acid conjugates linked with valine. Among the four bile acid prodrugs, the prodrug acyclovir valylchenodeoxycholate showed the highest affinity for human ASBT (hASBT) [257]. The cellular uptake of the prodrug showed substantially greater accumulation (16-fold) *in vitro* in COS-7 cells transfected with human ASBT (hASBT-COS cells). However, the oral bioavailability of acyclovir valylchenodeoxycholate only exhibited a 2-fold increase, probably due to hydrolysis of the prodrug in the proximal GIT before reaching the absorption site in the intestine [257]. Khatun et al. developed hydrophilic bile acid-conjugated self-assembled NPs to ameliorate the oral bioavailability of docetaxel. Hydrophilic taurocholic acid was used to modify the outer surface of the NPs, which increased the possibility of an interaction between docetaxel-loaded nanoparticles and the intestinal ASBT transporter. The results obtained from *in vivo* studies indicated that taurocholic acid-heparin-docetaxel (HDTA) NPs not only improved the oral bioavailability of docetaxel (from 0.8 % to 9.08 %) but also increased its antitumor activity; a significant reduction in tumor volume was observed in animals treated with HDTA NPs compared with mice treated with saline [258]. The same group further developed two taurocholic acid-modified DDSs (a taurocholic acid-chitosan-coated siRNA-gold nanoparticle complex and a hyaluronic acid-taurocholic acid conjugated siRNA/protamine nanocomplex) for the oral delivery of siRNA to efficiently treat colorectal liver metastasis [259,260]. Glycocholic acid, the most abundant component in human bile salts [261], was also selected as a ligand to modify the surface of the nanoparticulate system targeting ASBT. Kim et al. first demonstrated that glycocholic acid can

Table 5
Examples of targeting strategies towards intestinal transporters in oral drug delivery.

Transporters	Cargo	Targeting strategies	References
LAT1	Quindine	Val, Ile-quindine; prodrug	[279,293]
$ATB^{0,+}$	Acyclovir (ACV)	Valacyclovir	[236]
	Ganciclovir	Valganciclovir	[280]
	5-Fluorouracil	L-carnitine-PLGA NPs	[281]
ASTB	Acyclovir (ACV)	ACV-Val-CDCA; prodrug	[257]
	Akt2-siRNA	Taurocholic acid- chitosan coated Akt2-siRNA- gold nanoparticles complex	[259]
	Akt-siRNA	Hyaluronic acid-taurocholic acid conjugated siRNA/protamine nano-complex	[260]
	Curcumin (Cur)	Taurocholic acid-Cur loaded nanostructured lipid carriers (NLCs)	[294]
	Exendin-4 (Ex-4)	Chondroitin sulfate-g-glycocholic acid-coated and Ex-4-loaded liposomes	[263]
	Gabapentin	CDCA-alpha-benzyl-glu-gapapentin, CDCA-glu-gapapentin; prodrug	[295]
	Insulin	Deoxycholic acid-modified and insulin-loaded chitosan nanoparticles	[296]
	Ketoprofen	CDCA-Lys-ketoprofen; prodrug	[297]
	Docetaxel	Taurocholic acid-nanoparticles	[258,298]
	–	Glycocholic acid-solid fluorescent probe nanoparticles	[262]
	–	Dexoycholic acid-nanocomplex	[299]
MCT1	Carbenicillin	Carindacillin; prodrug	[300,301]
	Gabapentin	XP13512; prodrug	[275,302]
	Insulin	Butyrate-lipid-polymer hybrid nanoparticles	[276,277]
OATP	Tebipenem	Tebipenem pivoxil	[303]
OCTN2	Butyrate	Butyryl-L-carnitine	[304]
	Gemitabine	L-carnitine-succinic-gemcitabine (GSC), L-carnitine-hexylic-gemcitabine (GHC), L-carnitine-octanedioic-gemcitabine (GOC), L-carnitine-decanedioic-gemcitabine (GDC); prodrugs	[289]
	5-Fluorouracil	L-carnitine-PLGA NPs	[281]
	Paclitaxel	L-carnitine-PLGA NPs	[290]
SGLT1	Acyclovir (ACV)	beta-glucoside-ACV; prodrug	[305]
	p-nitrophenol (p-NP)	p-nitrophenyl beta-D-glucopyranoside (p-NPglc); prodrug	[284,285]
		p-nitrophenyl beta-disaccharide; prodrug	[306]
	Gly-Gly-Tyr-Arg, GGYR	alpha,beta-SAPG-GGYR; prodrug	[286]
	Quercetin	Quercetin 4'-beta-glucoside (Q4G); prodrug	[287]
SMVT	Acyclovir (ACV)	Biotin-ACV; prodrug	[271]
	Gabapentin	Gabapentin enacarbil (XP13512); prodrug	[269,275]
	Glucagon-like peptide-1 (GLP-1)	Biotin-GLP-1; prodrug	[270]
	Glucagon-like peptide-1 (GLP-1)	Biotin-PEG-GLP-1; prodrug	[307]
	R.I-K-Tat9	PEG:(R.I-Cys-K(biotin)-Tat9)$_8$; prodrug	[266]
	R.I-K-Tat9	R.I.-K (biotin)-Tat9	[265]
	Saquinavir (SQV)	Biotin-SQV; prodrug	[264]
	Salmon calcitonin (sCT)	Biotin-sCT; prodrug	[268]
SVCT1	Saquinavir (Saq)	Ascorbyl-succinic-Saq; prodrug	[267]
	Paclitaxel	Ascorbate-conjugated PLGA NP	[272]
PepT1	Acyclovir (ACV)	L-val-ACV; prodrug	[241,308–313]
		L-Glu-Sar-ACV; prodrug	[244,245]
		Val-PLA-PEG nanoparticles	[250]
	Cytarabine	5′-L-Val-cytarabine; prodrug	[314]
	Curcumin	Val-, Phe-polymeric micelles	[252]
	d-Amphetamine	lisdexamfetamine dimesylate (LDX)	[315]
	Decitabine (DAC)	5′-O-L-val-DAC, 5′-O-L-trp-DAC and 5′-O-L-phe-DAC; prodrug	[316,317]
	Dicyanomethylene-4H-pyran (DCM)	DCM-KPV	[256]
	Didanosine (DDI)	5'-O-L-val-DDI; prodrug	[242]
	Docetaxel (DTX)	L-Val-Val-; L-Val-Phe-PLGA-PEG nanoparticles	[253]
	1-(2',5' dimethoxyphenyl)-2-aminoethanol) (DMAE)	Gly-DMAE; prodrug	[318]
	Floxuridine	D-Val-floxuridine; produg	[319,320]
		5'-L-isoleucyl or 5'-L-val- floxuridine; prodrug	[321]
	Ganciclovir (GCV)	Tyrosine-val-GCV; prodrug	[322]
	Glucosamine	Gly-Val-glucosamine; prodrug	[246]
	Guanidine oseltamivir carboxylate (GOCarb)	L-val-GOCarb; prodrug	[240,323]
	L-alpha-methyldopa	L-Phe-L-alpha-methyldopa; prodrug	[324]
	L-dopa	L-Phe-L-dopa; prodrug	[243]
		D-Phe-L-dopa; prodrug	[325]
	Levovirin	5′-(L)-val-levovirin; prodrug	[326]
	Lopinavir (LVR)	Val-Val-LVR and Gly-Val-LVR; prodrug	[247]
	LY354740	Alanyl-LY354740 (LY544344); prodrug	[327,328]
	Oleanolic acid (OA)	Amino acid/dipeptide-OA; prodrug	[248,329]
	Oxytocin (OXY)	Val-PEG-PLA/PLGA NPs	[251]
	Pterostilbene (Pt)	Ile-Pt; prodrug	[330]
	Saquinavir (SQV)	Val-Val-SQV and Gly-Val-SQV; prodrug	[249]
	Zanamivir	L-val-zanamivir; prodrug	[331]

conjugate to the surface of solid fluorescent probe NPs. The modified NPs increased the oral bioavailability up to 47 %, which was achieved by combining the ASBT-mediated cellular uptake and chylomicron transport pathways [262]. The same group further developed glycocholic acid-modified liposomes for the oral delivery of exendin-4 via ASBT targeting, showing striking oral bioavailability (approximately 19.5 %) in rats. After a 4-week treatment with daily administration, exendin-4-loaded ASBT-targeting liposomes via the oral route

were found to be at least as efficient as the subcutaneous injection of exendin-4 solution *in vivo* in T2DM rats [263]. These studies demonstrated that the components (e.g., taurocholic acid and glycocholic acid) in human bile salts could be successfully decorated on the surface of DDSs as ligands via a specific ASBT route to increase the oral bioavailability of fragile drugs with low permeability since ASBT-targeting prodrugs are not stable in the harsh GI environment.

3.3. Sodium-dependent vitamin transporters (SMVT and SVCT1)

Sodium-dependent vitamin transporters (SMVTs) are mainly involved in the absorption of vitamins (e.g., lipoic acid, pantothenic acid and biotin) and can be found in large numbers in the proximal intestinal epithelia [264]. The interaction of biotin with SMVTs highly depends on the pH of the surrounding environment. Biotin has been exploited as a targeting ligand to develop SMVT-targeted prodrugs of poorly permeable therapeutics. Ramanathan et al. conjugated biotin with a nanopeptide (N-acetyl-L-Arg-L-Lys-L-Lys-L-Arg-L-Arg-L-Gln-L-Arg-L-Arg-L-Arg-L-Cys-NH_2, R.I.-K-Tat9) to exploit the intestinal SMVT transporter. R.I.-K-Tat9-biotin conjugates significantly increased transport *in vitro* in Caco-2 cell monolayers compared to R.I.-K-Tat9 alone [265]. PEG–biotin was synthesized, and these conjugates could also target the SMVT transporter [266]. Other peptide drugs (GLP-1 and sCT), antiviral drugs (acyclovir), anticonvulsant drugs (gabapentin) and antiretroviral drugs (saquinavir) in the form of biotin conjugates also exhibited enhanced permeability owing to their interaction with the SMVT transporter [267–271].

SVCT is predominantly responsible for the intestinal absorption of vitamin C (ascorbic acid, AA). Luo et al. demonstrated the ability of using an SVCT1-targeting prodrug for the oral delivery of saquinavir. The transport of SVCT1-targeted saquinavir was increased by approximately 5-fold *in vitro* in intestinal cell monolayers [267]. Ascorbic acid was also coated on the outer surface of paclitaxel-loaded PLGA nanoparticles (As-PLGA NPs) [272]. As-PLGA NPs with 20 % ascorbate conjugation showed higher uptake primarily through the caveolae-mediated pathway *in vitro* in Caco-2 cells. *In situ* perfusion and *ex vivo* biodistribution studies showed that after passing through the mucus layer, As-PLGA NPs interacted with the SVCT1 transporter and were further taken up by enterocytes, finally entering systemic circulation [272]. This is the only study exploiting the SVCT1-targeting approach for developing nanoparticulate systems to enhance the oral bioavailability of encapsulated drugs.

3.4. Monocarboxylate transporter MCT1

The monocarboxylate transporter (MCT1), present in both the apical and basolateral regions of intestinal epithelial cells, has predominant expression in the distal segment of the large bowel [273]. MCT1 mainly moderates the transport of unbranched monocarboxylates (such as SCFAs), and its substrates include β-hydroxybutyrate, acetoacetate, lactate and pyruvate [232]. The exploitation of MCT1 targeting has become an effective strategy for improving the oral absorption of some pharmacological therapeutics. Gabapentin, an anticonvulsant and analgesic drug, is primarily absorbed by the L-type amino acid transporter, which is a low-capacity solute transporter in the intestine, resulting in its limited oral absorption [274]. Cundy et al. produced a gabapentin prodrug (XP13512) by reversible modification of its amine group with an acyloxyalkylcarbamate moiety [275]. The targeting of MCT1 by ligand-decorated nanocarriers has also shown improved oral bioavailability of insulin [276]. Butyrate-decorated NPs, through their high affinity for the MCT transporter, elevated the transport of insulin up to 3.5-fold in Caco-2 cell monolayers and increased the oral bioavailability of insulin to 9.28 % in diabetic rats compared with that of undecorated nanoparticles [276]. The authors further disclosed the comprehensive transport mechanisms of these butyrate-modified NPs (Bu NPs) [277]. The results showed that Bu NPs actively bound to MCT1, and then most of the endocytosed Bu NPs were exocytosed both from the apical side back to the intestinal lumen and from the basal side into the bloodstream. Based on mechanistic studies of Bu NPs *in vitro*, studies further discovered that increasing the number of MCT1 cells in the basolateral membrane or increasing the surface hydrophobicity of the delivery system could facilitate the transport of Bu NPs.

3.5. Amino acid transporters (LAT1 and $ATB^{0,+}$)

Two amino acid transporters, $ATB^{0,+}$ and LAT1, have been studied as targets in oral drug delivery. They are predominately located on the luminal surface of the colon, and their expression levels are dramatically decreased in the jejunum and ileum. $ATB^{0,+}$, driven by Na^+/Cl^- gradients, has extensive substrates; it can transport most neutral and cationic amino acids. On the other hand, LAT1, unlike $ATB^{0,+}$, does not have such diverse substrate specificity; it is merely responsible for the transport of L-type large neutral amino acids presenting branched and aromatic side chains (e.g., valine) [236,278]. Amino acid-based prodrugs have exhibited great potential as targeted delivery systems to improve the cellular absorption of an antiarrhythmic agent (quinidine [279]) and an antiviral drug (ganciclovir [280]). Both $ATB^{0,+}$ and LAT1 are highly expressed in cancer cells. As an example, the expression levels of $ATB^{0,+}$ are significantly increased in colon cancer cells [281], and LAT1 is highly expressed in gastric tumors [282]. Only one study has demonstrated that $ATB^{0,+}$-targeted PLGA NPs could increase cellular uptake *in vitro* in Caco-2 cells compared with nontargeted NPs [281]. There is no example of an orally improved nanoparticulate system for LAT1. Addressing the potential of amino acid transporters ($ATB^{0,+}$ and LAT1) towards enhanced oral DDSs still needs to be further studied.

3.6. Sodium-coupled glucose transporter SGLT1 and organic cation/carnitine transporter OCTN2

SGLT1 is predominately located in the small bowel rather than in the large bowel [283]. Glycosylated molecules and glucose- and galactose-conjugated compounds have been suggested to enhance intestinal absorption via the SGLT1 transporter [284–287].

OCTN2, largely expressed in the apical membrane of enterocytes, transports carnitine and other organic cations across the epithelium [288]. Recently, OCTN2 has attracted attention as a target for increasing intestinal absorption. Wang et al. reported the development of gemcitabine prodrugs conjugated with L-carnitine via different lipophilic linkages. The developed prodrugs could significantly increase the cellular permeability and oral bioavailability of gemcitabine, among which the hexane diacid-linked prodrug dramatically increased its uptake by 15-fold across Caco-2 cells and its plasma absorption by 5-fold in rats. This study was the first to demonstrate that OCTN2 may represent a novel target for oral prodrug delivery [289]. They further exploited L-carnitine as a ligand to conjugate PLGA NPs for the oral delivery of paclitaxel [290]. PLGA NPs with a 10 % modification of L-carnitine exhibited the highest systemic absorption of paclitaxel in rats *in vivo*, and its intestinal absorption was predominantly via the lymphatic system [290]. Apart from OCTN2, $ATB^{0,+}$ is another transporter that is responsible for the transportation of carnitine but shows a relatively lower affinity compared to OCTN2 [291]. Both OCTN2 and $ATB^{0,+}$ exhibit significantly increased expression in colon cancer cells compared to normal colonocytes. Kou et al. conjugated L-carnitine with 5-fluorouracil-loaded PLGA NPs to develop a dual targeting nanoparticle, which could deliver 5-fluorouracil to overexpressed OCTN2- and $ATB^{0,+}$ in colon cancer cells [281]. In addition, OCTN2 is associated with other pathologies, such as IBD and diabetes [288]. Thus, OCTN2 might represent a novel and promising pharmacological target in oral drug delivery.

To date, over 400 membrane transporter proteins have been

identified in the human genome, and they are classified into two major superfamilies: 49 ABC transporters and 395 SLC transporters [292], while their targeting role in oral drug delivery has only been exploited for approximately 10 proteins (belonging to the SLC transporter superfamily), as reviewed above. These key transporters, located on both the apical and basolateral membranes of intestinal epithelium cells, represent excellent targets in oral drug delivery because they mediate nutrient–drug interactions. In recent years, the targeted-prodrug approach has been well developed in transporter-based oral delivery research, by which moieties are covalently conjugated to drug molecules, thereby selectively targeting certain transporters in the intestine. The developed targeted prodrugs, recognized by one or more well-known transporters in the GIT, offer a distinguished feasibility for increasing the selectivity and oral bioavailability of poorly permeable therapeutics. However, these transporter-targeted prodrugs still face large challenges, presenting limitations in the harsh GI environment and/or the expression and localization of transporters. Various transporter-targeting prodrugs might be hydrolyzed in the stomach and proximal intestine, resulting in inadequate absorption of the parent drugs. Some transporters are expressed throughout the whole intestinal tract but are expressed at relatively higher levels in certain intestinal segments, disease areas and specific cancer cells. Although it provides a long oral absorption window, the risk of toxicity from some therapeutics (such as anticancer drugs) will be largely increased because of the difficult accumulation of these prodrugs at specific sites and/or cells in the intestine. In recent years, nanoparticulate delivery systems have also accomplished some achievements in the intestinal transporter-targeting field. NPs enhance the efficacy of orally administered drugs not only by increasing solubility, avoiding acidolysis and hydrolysis and controlling release in the GIT but also by further modifying specific substrates with drug carriers as ligands to target the transporters expressed in certain segments of the intestine. The examples we reviewed showed that nanoparticulate delivery systems conjugated with ligands of one or more specific substrate represent promising potential towards orally administered therapeutics. Nevertheless, the data on how relevant transporters mediate the intestinal absorption of targeted NPs are still limited, and more studies are required to unveil the behaviors of NPs, such as the comprehensive transport mechanism of transporter-targeting NPs. This would provide more information for the rational design of transporter-targeting NPs.

4. Conclusion

DDSs targeted to the gastrointestinal epithelium are one of the most exploited strategies in the oral delivery of drugs. This strategy has been greatly exploited to increase the bioavailability of poorly water-soluble drugs or biologics or to increase the accumulation of the drug delivery system at the site of interest, as mentioned in the sections above. Advanced formulations, via unique characterizations, promising strategies and potential ligands, can be used to target different sites, including different cells, transporters and specific disease sites, throughout the whole GIT. However, many receptors/cells/strategies remain unexplored and represent promising therapeutic targets for successful oral drug delivery systems. There are important gaps in knowledge regarding the interaction of the DDS with the gastrointestinal barrier that hinder the evolution of oral targeted drug delivery. As in most of the studies described herein, the mechanisms by which the drug delivery system interacts with each cell/transporter is still unknown. This is a key limitation in the evolution of oral drug delivery.

The examples we reviewed showed that cell receptor/transporter-mediated NPs with the further modification of one or more ligands represent potential formulations for applications towards orally administered drugs. However, it should be fully taken into consideration that the examples described within this review correspond to preclinical studies and that the real clinical value of these strategies remains uncertain. Current studies in oral targeting strategies mostly are proof-of-concept, obtaining promising results in preclinical phases but not reaching the clinics. Although we have shown good experimental results by targeting different intestinal cells and segments via specific receptors or transporters, it is essential to consider the real interest in the targeting of those cells and sites in a foreseen clinical translation. From the physiological point of view, enterocytes have an absolute advantage in improving the oral bioavailability of poorly absorbed drugs since they represent the most abundant cell type in the gut. They are mainly responsible for the absorption of nutrients from the lumen, and express various vital receptors and transporters. This does not mean that the targeting of other intestinal cells has no clinical value. On the contrary, the studies regarding different cell targeting strategies provide a deep understanding on how to overcome the intestinal barriers via oral route. In addition to the improved bioavailability via enterocytes-targeting, current studies have also demonstrated the contribution of M cells to oral vaccine development and the potential use of L cells-targeting for the treatment of diabetes. Furthermore, once the formulations are administered, it is impossible to only deliver the administered cargoes into specific cells due to the direct interplay with different types of intestinal cells. The studies regarding different cell targeting summarized here highlight the advantages of those targeting approaches. Depending on the characteristics of the disease to be treated and the type of drug to be selected, we can select single or combined strategies towards cell targeting in future applications. Moreover, considering a foreseen clinical application, one must also be realistic regarding the manufacture of these DDSs. Most of these systems are available in small-scale laboratory batches, but their manufacturing on a large scale would be too expensive and complicated.

Another important conclusion that can be drawn from these examples is that we might not need to engineer the surface of the drug delivery system in each case for it to be effective, and we might be able to exert biological effects and/or targeting capacities simply by exploiting the physicochemical properties of the DDS. However, the optimal physicochemical properties (e.g., surface charge, particle size) would vary depending on the cell type or the transporter being targeted, and these properties would have to be established for each and every example described herein.

In any case, recent studies on the targeting of L cells and the targeting of specific cell receptors/transporters prove that there is still room for the development and improvement in the oral drug delivery field towards more effective targeted DDSs.

Declaration of competing interest

The authors declare no competing interests.

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