Advances in the Molecular Mechanisms of Rumen Epithelial Cell Proliferation and Substance Transport: Postprint

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Abstract

The rumen, as a unique digestive organ of ruminants, plays a crucial role in the digestion and utilization of dietary nutrients. Over the past five decades, extensive research has been conducted on rumen epithelial cell development and nutrient transport, with a particular focus on elucidating the molecular mechanisms underlying rumen epithelial proliferation and the regulatory pathways of associated transport proteins. For instance, insulin-like growth factor (IGF) and epidermal growth factor (EGF) are involved in regulating glucose transport, while sodium-hydrogen exchanger (NHE), monocarboxylate transporters (MCTs), and G protein-coupled receptors (GPR) participate in the transport of short-chain fatty acids (SCFA) across rumen epithelial cells. Nevertheless, our understanding of the intrinsic mechanisms governing rumen development remains very limited. This review summarizes recent research advances in the molecular mechanisms of rumen epithelial cell proliferation and nutrient transport in ruminants, which holds significant importance for further comprehending the process of rumen epithelial development and establishing optimal nutritional strategies for ruminants.

Full Text

Molecular Mechanisms of Ruminal Epithelial Cell Proliferation and Substance Transportation

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Abstract

The rumen is a unique digestive organ in ruminants that plays a critical role in dietary nutrient digestion and utilization. Over the past five decades, extensive research has focused on ruminal epithelial development and substance transport, particularly exploring the molecular mechanisms governing ruminal epithelial proliferation and the regulatory pathways of related transporters. For instance, insulin-like growth factor (IGF) and epidermal growth factor (EGF) participate in regulating glucose transport, while sodium-hydrogen exchanger (NHE), monocarboxylate transporters (MCTs), and G protein-coupled receptors (GPR) are involved in short-chain fatty acid (SCFA) transport across ruminal epithelial cells. Nevertheless, our understanding of the intrinsic mechanisms underlying rumen development remains limited. This review summarizes recent advances in the molecular mechanisms of ruminal epithelial cell proliferation and substance transport, which is crucial for further understanding ruminal epithelial development and establishing optimal nutritional strategies for ruminants.

Keywords: ruminants; ruminal epithelium; cell proliferation; substance transportation; molecular mechanism

1. Structural and Functional Characteristics of Ruminal Epithelium

In adult ruminants, the rumen and reticulum account for approximately 70% of total digestive tract volume. The ruminal wall comprises three layers: mucosa (ruminal epithelium), serosa, and muscularis [1]. The ruminal epithelium is a stratified structure consisting of four distinct layers, from the mucosal to serosal surface: the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. Basal layer cells are responsible for epithelial renewal and damage repair. Additionally, the basal and spinous layers contain abundant mitochondria that participate in volatile fatty acid (VFA) metabolism and ketogenesis. The granular layer features numerous tight desmosomal junctions that primarily prevent substances from entering the ruminal epithelium via diffusion. The cornified layer directly contacts ruminal contents and contains abundant keratin, serving as a barrier between the ruminal environment and external milieu. This multi-cellular architecture forms the ruminal epithelial barrier, which regulates nutrient absorption and prevents translocation of toxic substances and microorganisms into the bloodstream [2]. The ruminal epithelium hosts a large microbial population, including bacteria, protozoa, fungi, and archaea [3], which are intimately associated with host digestion, nutrient absorption, and immune function.

The rumen of newborn ruminants is sterile, but microorganisms appear within 1-2 days after birth. Microbial colonization induces changes in host nutrient digestion and metabolism. As young ruminants transition from milk-based to feed-based digestion, the composition and abundance of ruminal microorganisms also change accordingly [4]. In mature rumen and reticulum, approximately 75%

of total VFA are absorbed under normal conditions [5], with VFA serving as the primary energy source for adult ruminants. Acetate and butyrate are converted to ketone bodies in the ruminal epithelium, while propionate is metabolized to lactate and glucose. Compared to adults, young ruminants have underdeveloped rumen with different dominant microbial populations, and their ruminal epithelial basal and granular layers lack ketogenic capacity. Consequently, glucose serves as their main energy source. However, by 30 days of age, VFA metabolism in calf ruminal epithelial cells reaches 40% of adult levels. In vivo studies have shown that glucose utilization in lambs decreases by approximately 90% from 2 days to 6 months of age, while utilization of butyrate and lactate gradually increases [6], a finding confirmed by in vitro studies [5]. Ruminal epithelial cells lack ruminal ketogenic enzymes, which are likely produced in mitochondria-rich basal layer cells [7]. Ruminal ketogenesis in ruminants primarily depends on mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (the rate-limiting enzyme) and acetoacetyl-CoA thiolase [8]. HMG-CoA synthase and acetoacetyl-CoA thiolase serve as markers of ruminal development, with their mRNA expression levels gradually increasing from 0 to 84 days of age, reaching adult levels by day 84. As age advances and young ruminants transition from milk to forage and feed, ruminal microbial composition and abundance continuously change, eventually stabilizing in adulthood.

2.1 Insulin Family

The insulin family comprises IGF-1, IGF-2, three receptors (IGF-1R, IGF-2R, and insulin receptor protein [IGF/InsR]), and six high-affinity binding proteins [insulin-like growth factor binding proteins (IGFBP) 1-6] [9].

IGF-1 promotes DNA and RNA synthesis and cell proliferation, facilitating cell cycle progression from G1 to S phase [10]. IGF-1 participates in cell proliferation regulation through several known signaling pathways (Figure 1 [Figure 1: see original paper]): the phosphatidylinositol-3-kinase (PI3K)/serine/threonine kinase (AKT) pathway (AKT pathway), the Ras/Raf/MEK/ERK pathway (ERK pathway), and the 14-3-3 protein/Raf-1 pathway. The AKT and ERK pathways control cell proliferation: ERK accelerates G1 progression and promotes cyclin D1 expression, while AKT inhibits cyclin D1 degradation, and the 14-3-3/Raf-1 pathway promotes proliferation by inactivating the pro-apoptotic protein BAD [11]. IGF-1 exerts physiological functions by binding to IGF-1R. Studies have shown that IGF-1 promotes ERK phosphorylation in goat ruminal epithelial cells, with cyclin D1 protein expression significantly elevated following IGF-1 treatment compared to controls [12], confirming the role of the ERK pathway.

Dietary nutrient levels affect IGF-1 expression, with high-nutrient diets promoting IGF-1 and IGF-1R expression in a coordinated manner, increasing cyclin D1 and CDK4 protein expression and accelerating cell cycle progression to promote ruminal epithelial proliferation [12]. Dietary amylose/amylopectin ratios also in-

fluence IGF-1 and IGF-1R expression in ruminal epithelial cells [13], possibly due to different rumen passage rates altering VFA proportions, though this requires further verification.

The insulin receptor (InsR) mediates insulin and IGF-2 signals to affect cell proliferation and differentiation. IGF-2 binding to InsR' s α-subunit induces autophosphorylation of the β-subunit on tyrosine residues, transmitting signals to insulin receptor substrate-1 (IRS-1) and subsequently activating PI3K and ERK to influence multiple signaling pathways [14]. Few studies have reported on InsR in ruminal epithelium, though Hou [15] demonstrated that isobutyrate promotes InsR expression. IGF primarily regulates ruminal epithelial proliferation through the IGFBP family. IGFBP-3 and IGFBP-5 have been extensively studied with opposing functions: upregulated IGFBP-3 expression promotes cell proliferation [16]. Dietary energy level, structure, and type affect IGFBP-3 expression, with high-energy diets significantly increasing IGFBP-3 mRNA expression [11]. Steele et al. [17] found that grain-based diets downregulated IGFBP-3 and upregulated IGFBP-5, whereas Weng [18] reported that grain diets upregulated both IGFBP-3 and IGFBP-5 to promote ruminal epithelial proliferation, with the underlying mechanism remaining unclear. Increasing dietary neutral detergent fiber (NDF)/starch ratios significantly decreased IGFBP-5 expression while increasing IGFBP-6 expression, without significantly affecting IGFBP-3 [19]. IGFBP-6 binds IGF-2 to inhibit cell proliferation, though the specific mechanism requires further investigation. Additionally, increased ruminal butyrate content downregulates IGFBP-3 expression to promote ruminal epithelial proliferation [20]. Feeding regimens also affect IGFBP-3 and IGFBP-5 expression: 28-day weaning promoted lamb rumen development and IGFBP-5 expression, possibly related to high SCFA content in early-weaned lambs or solid feed intake stimulating rumen development. However, IGFBP-3 expression positively correlated with ruminal papillae development [21], contradicting previous findings and warranting further investigation.

IGF-1 participates in glucose transport regulation, with more studies in non-ruminants than ruminants. Two transporters are known to be involved: sodium/glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2). Ren [22] found that IGF-1 promotes facilitative glucose transporter protein (Glut) expression in human cancer research. During glucose transport, IGF-1 binding to IGF-1R enhances GLUT1 expression, significantly increasing glucose transport capacity [23]. However, Ader et al. [24] reported minimal glucose transport capacity of GLUT2 in sheep, and IGF-1 effects on glucose transport remain unreported in ruminants.

Figure 1 The sketch map of signal transduction pathways of IGF-1 [11-12]

2.2 EGF

EGF is a potent growth factor that promotes gastrointestinal development, repairs damaged mucosal tissue, and enhances nutrient absorption and metabolism. EGF promotes cell proliferation, with colostral EGF stimulating gastrointestinal development in young animals and promoting DNA and protein synthesis in intestinal wall cells. EGF also promotes colon cell proliferation and RNA and protein synthesis in humans [25], and in vitro studies confirm EGF promotes ruminal epithelial cell proliferation [26]. Bedford et al. [27] found that EGF first binds to specific membrane receptors to transmit extracellular signals into cells, activating transcription factors for cell proliferation. EGF receptors include receptor tyrosine kinase (TPK) and GPR, which cooperate in signal transduction. TPK is critical for EGF-induced cell proliferation, mediating primarily through the JAK-STAT and TPK-Ras-MAPK pathways, while GPR signals through GPR-Ras-cdc42-JNK and GPR-Ras-MAPK pathways [28]. Ras GTPase transmits signals from upstream to downstream molecules, with MAPK belonging to the protein kinase family. Zhang et al. [29] demonstrated that EGF regulates HIF-α, Bax, and Bcl-2 mRNA expression in yak cumulus cells to control apoptosis, where Bax inhibits apoptosis, Bcl-2 is anti-apoptotic, and HIF-α promotes neuronal apoptosis by inhibiting Bcl-2 expression. Whether EGF regulates ruminal epithelial cell apoptosis through these mechanisms remains unknown. EGF promotes PCNA expression in porcine small intestine [30]; PCNA is a cyclin that promotes DNA synthesis and cell proliferation [31], though PCNA expression in ruminant ruminal epithelium and whether EGF promotes proliferation through PCNA remain unclear. EGF is also closely related to glucose transport: upregulated SGLT1 expression enhances glucose transport [32], and EGF promotes SGLT1 expression in weaned piglets to facilitate glucose transport [33]. SGLT1 expression and glucose absorption via SGLT1 have been confirmed in rumen [34], but EGF regulation of SGLT1 expression in rumen remains unreported.

2.3 NHE

NHE is a transmembrane protein present in all eukaryotic cells. To date, ten NHE isoforms (NHE-1 to NHE-10) have been identified. NHE regulates intracellular pH, maintains cell volume, influences ion transport, and controls cell proliferation and apoptosis. NHE facilitates equal exchange of extracellular Na+ for intracellular H+ [35] (Figure 2 [Figure 2: see original paper]), thereby maintaining intracellular pH homeostasis. Studies suggest NHE regulates cell proliferation by controlling the cell cycle: NHE-1 activation accelerates G2/M phase progression, while NHE-1 deficiency causes significant S-phase delay and cell division arrest [36]. Metabolic enzymes function optimally under alkaline pH; NHE activation creates an alkaline intracellular environment that enhances metabolic enzyme activity [37]. Key factors for cell proliferation, including protein, DNA, and RNA synthesis, increase under alkaline conditions, promoting

active cell proliferation. Na+/H+ ATPase is also associated with cell proliferation. NHE-1 has been extensively studied in tumors, with Liu et al. [38] demonstrating its involvement in esophageal cancer KYSE-70 cell proliferation in vitro. Recently, NHE-1 has become a research focus in ruminant foregut studies, showing high expression in ruminal epithelium [39]. Ruminal epithelial cells pump out H+ via NHE-1, creating an alkaline intracellular environment favorable for chemical reactions and accelerated proliferation. Feeding regimens affect Na+/H+ ATPase gene expression, promoting cell proliferation through enhanced enzyme activity. Increased dietary concentrate elevates VFA content and decreases ruminal pH, with VFA content and pH regulating NHE-1 and NHE-3 expression [40] but not significantly affecting NHE-2 [41], suggesting other regulatory factors for NHE-2 that require further investigation. Rat studies show that NHE-1 blockade significantly reduces PCNA expression [42], though PCNA expression in ruminant ruminal epithelium and NHE-1 effects on PCNA remain to be elucidated. The molecular mechanisms of NHE-1 regulation of ruminal epithelial proliferation are not fully understood and require further study.

Figure 2 The sketch map of NHE and MCT transporting SCFA [35]

2.4 MCTs

MCTs belong to the monocarboxylate transporter family responsible for transmembrane transport in mammalian cells. Among fourteen identified family members, only MCT1, MCT2, and MCT4 possess monocarboxylate transport function [39]. Current MCT research focuses on humans and monogastric animals. In human tumor studies, normal cells exhibit growth inhibition due to accumulation of lactate, propionate, and other metabolites, whereas tumor cells do not. Alves et al. [43] found abundant MCT expression in tumor cells, primarily involved in lactate transport. The specific molecular mechanisms of MCT-promoted tumor cell proliferation may involve lactate and pyruvate transport to prevent intracellular accumulation and promote tumor cell survival. Studies have confirmed MCT1 and MCT4 expression in ruminant ruminal epithelium [43], with MCT1 primarily located in basal and spinous layers and MCT4 in cornified and granular layers. MCTs are crucial for VFA transport in ruminants, as VFA represent the primary energy source. VFA absorption involves two processes: (1) uptake across apical membranes, and (2) basolateral efflux. VFA enter ruminal epithelial cells via different routes: at low pH, proton-coupled VFA passively diffuse across cell membranes, with absorption rates determined by pH at the stratum corneum apical membrane; at high pH, the ion cotransporter MCT1 cotransports H+ and monocarboxylate anions in a 1:1 ratio [44] (Figure 2). SCFA promote ruminal development [45], suggesting SCFA may enhance MCT1 expression through specific signaling pathways. Increasing dietary concentrate from 10% to 35% significantly elevated MCT1 and MCT4 expression in goat ruminal epithelium [46]. While increased concentrate proportion

decreases ruminal pH and increases SCFA content, in vivo experiments show pH and SCFA content co-regulate MCT1 and MCT4 expression, but in vitro studies show no significant effects of pH and SCFA on MCT4 expression [43], indicating that dietary regulation of MCT4 expression requires further investigation.

2.5 GPR

GPR represents one of the most active research areas in life sciences. GPR on cell surfaces couples with GTP-binding proteins and binds various extracellular ligands to regulate diverse physiological responses. VFA are important energy sources for ruminants, with GPR41 and GPR43 being the only confirmed specific SCFA receptors identified to date. Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other SCFA [47]. These receptors sense fatty acids in the digestive tract; GPR41 knockout mice show reduced intestinal fatty acid absorption, while microbiota-depleted GPR41 knockout and normal mice exhibit no weight differences, indicating GPR41 regulation occurs through SCFA produced by gut microbial fermentation [48]. GPR41 and GPR43 are closely associated with the MAPK signaling pathway, which includes stress-activated protein kinase p38, JNK, and ERK. MAPK is a crucial signaling pathway regulating cell differentiation and proliferation, primarily responsible for phosphorylating and downregulating the anti-apoptotic protein Bcl-XL. MCT1 expression colocalizes with GPR41 and GPR43 [49], suggesting these receptors may regulate MCT1 through similar pathways. Additionally, GPR41 affects the cell cycle, participating in G1-to-S phase transition [50]. Recently, GPR41 and GPR43 have become research hotspots in ruminant ruminal development, with expression confirmed in ruminal epithelium. Notably, GPR43 is present in bovine ruminal epithelium but not in pancreatic islets [51], providing theoretical basis for VFA as signaling molecules that directly mediate ruminal epithelial cell proliferation.

Dietary effects on GPR41 and GPR43 expression have attracted widespread attention. High-concentrate diets promote butyrate production and GPR43 expression, thereby stimulating ruminal epithelial proliferation [24]. Butyrate is a gene transcription regulator that may activate MAPK and promote cell proliferation by downregulating Bcl-XL expression, though the mechanism of high-concentrate diet-induced GPR43 expression requires further verification. The specific regulatory mechanisms of GPR41 and GPR43 in ruminal epithelial proliferation remain unclear, and few studies have examined dietary nutrient level and feeding regimen effects on their expression, representing an excellent starting point for ruminal development research.

Over the past decade, ruminal development has been a focal point in ruminant nutrition research. Current studies primarily concentrate on the molecular mechanisms of IGF, NHE, EGF, MCTs, and GPR in regulating ruminal epithelial

cell proliferation and substance transport, with substantial evidence demonstrating that dietary nutrient levels and feeding regimens affect expression of these genes. For IGF research, the IGFBP family represents a worthwhile target for studying epithelial cell proliferation regulation. For NHE and EGF research, whether PCNA is expressed in ruminant ruminal epithelium and whether EGF and NHE-1 affect PCNA expression remain to be elucidated. GPR research in ruminants is just beginning, with the specific regulatory mechanisms of GPR41 and GPR43 still unclear; investigating how dietary nutrient levels and feeding regimens affect GPR41 and GPR43 expression may provide a breakthrough. MCT research has primarily focused on monogastric animals, with incomplete understanding of their cell proliferation regulatory mechanisms and uncertain relevance to ruminants. Since MCTs are closely associated with SCFA transport —the main energy source for ruminants—and SCFA promote ruminal epithelial proliferation, investigating MCTs as an entry point to deeply study SCFA transport and its mechanisms in promoting ruminal epithelial proliferation holds significant importance for ruminant nutrition research.

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