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Review

Intestinal redox biology and oxidative stress

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ABSTRACT

The intestinal epithelium sits at the interface between an organism and its luminal environment, and as such is prone to oxidative damage induced by luminal oxidants. Mucosal integrity is maintained by the luminal redox status of the glutathione/glutathione disulfide (GSH/GSSG) and cysteine/cystine (Cys/CySS) couples which also support luminal nutrient absorption, mucus fluidity, and a diverse microbiota. The epithelial layer is uniquely organized for rapid self-renewal that is achieved by the well-regulated processes of crypt stem cell proliferation and crypt-to-villus cell differentiation. The GSH/GSSG and Cys/CySS redox couples, known to modulate intestinal cell transition through proliferation, differentiation or apoptosis, could govern the regenerative potential of the mucosa. These two couples, together with that of the thioredoxin/thioredoxin disulfide (Trx/TrxSS) couple are the major intracellular redox systems, and it is proposed that they each function as distinctive redox control nodes or circuitry in the control of metabolic processes and networks of enzymatic reactions. Specificity of redox signaling is accomplished in part by subcellular compartmentation of the individual redox systems within the mitochondria, nucleus, endoplasmic reticulum, and cytosol wherein each defined redox environment is suited to the specific metabolic function within that compartment. Mucosal oxidative stress would result from the disruption of these unique redox control nodes, and the subsequent alteration in redox signaling can contribute to the development of degenerative pathologies of the intestine, such as inflammation and cancer.

Contents

Abbreviations: Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; Trx, reduced thioredoxin; TrxSS, oxidized thioredoxin.

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1. Organization of the intestinal epithelium and regenerative potential

1.1. Structural and functional organization

The mammalian gastrointestinal epithelium is capable for self-renewal every 4–5 days and is one of the highest proliferative tissue in an organism [1]. The small intestinal epithelium is extensively folded into crypts (of Lieberkühn) and villi, a structural feature that maximizes the absorptive surface area for nutrients, electrolytes and water. Within the epithelium the five major cell types are derived from an absorptive lineage, i.e., enterocytes, or a secretory lineage, i.e., mucus-secreting globlet cells, hormone-secreting enteroendocrine cells, Paneth cells, and tuft cells. The multi-potent intestinal stem cell (ISC) and progenic transit-amplifying cell reside in the crypt and proliferate into progenitor cells that migrate to the villus tip. The stem cell niche is supported by surrounding stromal or mesenchymal cells known as the lamina propria that regulates stem cell behavior through secretion of growth factors and cytokines [2]. During crypt-to-villus transition, the progenitor cells differentiate into goblet, enteroendocrine, tuft, or epithelial (enterocyte) cells; Paneth cells differentiate and remain within the crypt [3]. The villus tip enterocytes at 4–5 days post-differentiation undergo spontaneous apoptosis and are shed into the intestinal lumen, thus completing one renewal cycle (Fig. 1). Likewise, colonic crypts house proliferative cells and differentiated cells reside at epithelial surfaces, but the colonic epithelium is distinctly devoid of villi and Paneth cells.

Enterocytes are most abundant, at >80%, and they function in apical nutrient and water absorption. Secretory goblet cells comprise ~16–50% of the small intestinal and colonic epithelium, respectively. The functions of goblet cells include the secretion of mucus and a supportive environment for the gut microbiota while the other secretory enteroendocrine, Paneth, and tuft cells each perform essential functions in gastrointestinal hormone secretion, host defense, and bicarbonate secretion, respectively [4,5]. Tuft cells also uniquely express cyclooxygenase 1 and 2 (COX1 and COX2), which suggests their involvement in intestinal pathobiology [4]. The gastrointestinal tract is the hub for the gut-associated lymphoid tissue (GALT) [6], organized as Peyer's patches scattered along the intestine and are surrounded by a specialized follicle-associated epithelium (FAE). The FAE contains invaginated microfold cells (M cells), a class of specialized enterocytes which function in luminal antigen presentation to the dendritic cells, B- and T-lymphocytes within the lamina propria, and trigger an immune response [7].

The sequential processes of proliferation, migration, differentiation, and apoptosis within the intestinal epithelium are dynamic. At base crypts, asymmetric division of ISCs gives rise to two daughter cells, one remaining as a stem cell (self-renew) at the base, and the other becomes a transient amplifying cell that undergo differentiation (Fig. 1A). The "stemness" of ISCs is maintained by signals from adjacent Paneth cells. Wnt3, Notch ligand Dll4, and EGF signals support ISC growth but also limit ISC number [8], thereby maintaining a define number of crypt ISCs. ISC cell number is also regulated by mesenchymal cell-derived bone morphogenic proteins (BMP) which inhibit ISC proliferation [9].

1.2. Signaling of cell proliferation and differentiation

The signaling mechanisms that govern ISC proliferation and differentiation into mature intestinal epithelial cells are incompletely understood. Among the key candidates are the Wnt/β-catenin, Notch, and BMP pathways. The Wnt/β-catenin pathway is known to drive ISC proliferation and renewal within the intestinal crypt, participating in the maintenance of stem/progenitor cells, inhibition of stem cell differentiation, regulation of crypt-to-villus cell migration, early development of the secretory lineage, and terminal differentiation of Paneth cells [10,11]. A highly expressed Wnt-activated gene, *Lgr5* (*leucine-rich repeat containing G protein-coupled receptor 5*) in ISCs under basal conditions is a widely accepted stem cell marker [12]. The maintenance of ISCs in an undifferentiated, proliferative state is further supported by Notch signaling while BMP-dependent suppression of Wnt signaling promotes ISC quiescence [13].

Crypt-to-villus migration of progenitor cells is characterized by a decrease in Wnt signaling. Concurrent gradient increase in BMP activation was associated with increased cell quiescence and differentiation [14]. Cell fate decision between differentiating into a secretory or an absorptive lineage is the function of the Notch signaling pathway [15] (Fig. 1B) wherein depletion of *Hes-1* (*hairy/enhancer of split 1*), a direct Notch target gene, was associated with excessive number of goblet, enteroendocrine, and Paneth cells [16]. Conversely, the *Atoh1* (*Atonal homologue 1*) gene, which is repressed by Hes-1 transcription factor, is required for progenitor cell differentiation into a secretory lineage; reportedly all intestinal secretory cell types are derived from a single *Atoh1*-dependent secretory progenitor [17]. The finding that Atoh1 expression was influenced by Wnt signaling [15] suggests an interaction between the Wnt and Notch pathways. Other transcription factors, Gfi1, *Neurogenin3* (*Neurog3*), *SAM pointed domain containing Ets transcription factor* (*Spdef*), and *SRY-box containing gene 9* (*Sox9*), support differentiation into specific secretory cell types (Fig. 1B). Gfi1 represses *Neurog3* and is critical for progenitor cell-to-Paneth/goblet cell differentiation [18]. *Spdef* promotes terminal differentiation of goblet cells and maturation of Paneth cells [19], whereas *Sox9* is necessary for Paneth cell differentiation [20]. Tuft cell differentiation is modulated by the Lgr5-expressing ISC which acquired secretory characteristics in an Atoh1-dependent way [4]. While the mechanisms for M cell differentiation are yet unknown, these cells have been shown to derive from Lgr5 progenitor cells [21].

2. Redox biology of the intestine

2.1. Concept of cellular redox environment

The glutathione (GSH), cysteine (Cys), and thioredoxin (Trx) couples are the major cellular redox systems in cells [22], and the cellular redox state of the individual couples is described by its inter-convertible reduced and oxidized forms, i.e., GSH/GSSG, Cys/CySS, or Trx/TrxSS. The collective product of their reducing potential and reducing capacity constitute the intracellular redox environment [23]. The ratio of GSH-to-GSSG approximates the intracellular redox environment given the large cellular GSH pool size [23], and the tendency of GSH for electron donation or acceptance is defined by its redox potential, E_h. Under physiological conditions, E_h for GSH/GSSG is between −260 and −200 mV [24].

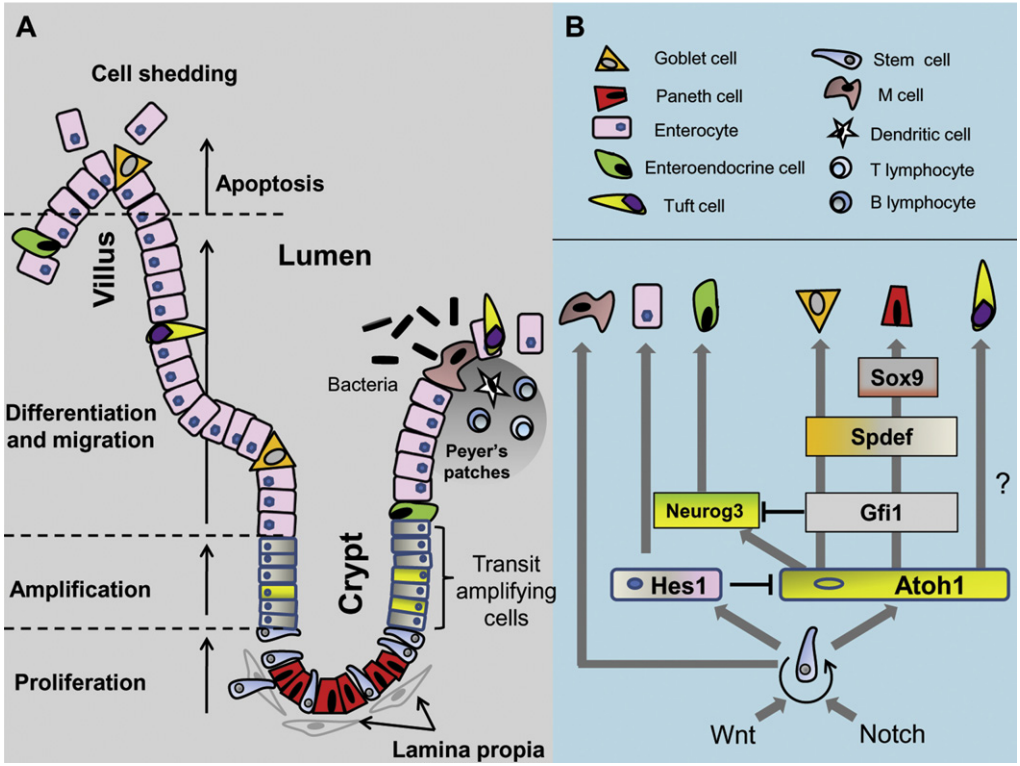

Fig. 1. Structural organization (A) and epithelial differentiation (B) in the small intestine. (A) The intestinal epithelium is organized into the villus and crypt regions each containing specialized cell types with distinct functions. Within the crypt, intestinal stem cell (ISC) progenitors differentiate into absorptive or secretory lineages along the crypt-to-villus axis. Differentiated Paneth cells remain in the crypt. Gut immune response is mediated by specialized M cells from GALT in Peyer's patches. Apical enterocytes undergo apoptosis and are shed into the lumen. (B) Intestinal self-renewal and ISC proliferation is regulated by signals from Paneth cells and the surrounding lamina propria. Notch-dependent activation of *Hes1* or *Atoh1* genes respectively determines the absorptive and secretory lineages. The mechanisms of ISC-to-M cell or Atoh-1-dependent Tuft cell differentiation are unknown.

An emerging view is that in biologic systems, the GSH/GSSG redox couple, together with those of Trx/TrxSS, and Cys/CySS serves as distinctive redox control nodes or circuitry in the redox control of cellular metabolism and growth [24]. The fact that each of the redox couples exists far from equilibrium and can function as on-off sulfur switches support such a mode for independent regulation of single protein or protein sets in normal physiology [25]. This viewpoint provides a novel metabolic framework to redefine cellular oxidative stress as the disruption of biological redox signaling events [22] as opposed to the classical view of oxidative stress as simply an imbalance between pro- and anti-oxidant systems [26]. The extent to which the mechanisms of sulfur switches and redox circuitry control intestinal cell function and growth is an open question.

2.2. Cellular redox systems and compartmentation

2.2.1. Glutathione and GSH-dependent enzymes

GSH is present in millimolar concentrations in the intestinal epithelium [27–29], a level that is similar to other cells types (2–10 mM) [30]. Intracellular GSH exists as the biologically active reduced-thiol form, and its oxidation to GSSG is oft associated with oxidative stress. Mucosal GSH redox homeostasis is maintained by de novo synthesis [31], regeneration from GSSG [32], and GSH uptake [28,33]. Mucosal GSH uptake across the apical membrane occurs independently of intracellular GSH synthesis [31], and is stimulated by monovalent cations [33,34], a characteristic that is shared by renal proximal tubular cells [35]. An important aspect of intestinal GSSG reduction is the supply of NADPH for the function of the GSH redox cycle [36].

Compartmental distribution of GSH likely exists within all cell types, including enterocytes. Cellular GSH is distributed among

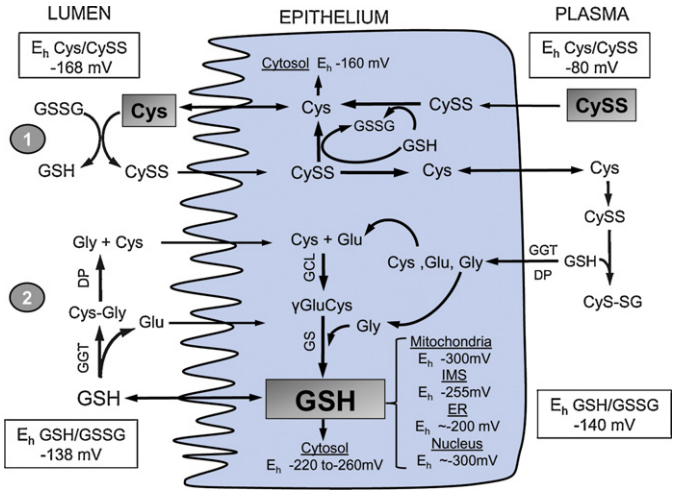

Fig. 2. Contribution of the Cys/CySS and GSH/GSSG systems to intestinal redox homeostasis. At intestinal brush-border the Cys/CySS shuttle (1) and γ-glutamyl cycle (2) support luminal thiol/disulfide homeostasis. Within the intestinal epithelium, CySS reduction and de novo GSH synthesis maintain intracellular E_h of the Cys/CySS and GSH/GSSG couples, respectively. Cytosolic GSH is exported or compartmentalized within subcellular compartments as distinct GSH redox pools. CySS predominates within plasma, Cys status is controlled by thiol/disulfide exchange with liver-derived GSH, and CyS-SG is hydrolyzed to constituent amino acids for enterocyte re-uptake. Shaded boxes for luminal Cys, cellular GSH, and plasma CySS represent the major determinant of the E_h in these compartments. Dietary GSH or Cys sources and basal-lateral GSH efflux or influx to-and-from plasma are not shown for purposes of simplicity.

mitochondria, endoplasmic reticulum (ER) and nucleus as distinct GSH redox pools [37] (Fig. 2). GSH levels are high in the mitochondrial, cytosolic and nuclear compartments with relatively reduced with E_h, between −260 mV and −300 mV [24]. In contrast, the ER matrix exhibits E_h between −170 mV and −205 mV [38]. The vast difference in redox potential within the different organelles is well suited to the specific biological or metabolic function within that compartment. For example, the oxidized environment of the ER supports proper folding of nascent proteins [39]. A GSH/GSSG E_h of −255 mV in the mitochondrial intermembrane space (IMS) supports disulfide bond formation of imported cytosolic proteins [40], even though the matrix exhibits a more reduced GSH E_h (∼300 mV) [41]. While matrix GSH balance is achieved through carrier-mediated cytosol-to-mitochondria GSH import [42], it remains unclear how the IMS maintains an oxidized E_h despite free access of cytosolic GSH through porin channels [43]. Cytosolic-nuclear GSH interaction is dynamic, notably during cell cycle wherein nuclear GSH increased 4-fold [44]. An unresolved dilemma is the supposed notion that nuclear GSH is maintained independently from that of cytosolic GSH [24] despite the fact that cytosol-to-nuclear GSH import occurs by passive diffusion through nuclear pores [45].

Intra-intestinal antioxidant defense is mediated by GSH-dependent enzymes that are compartmentalized within the cytosol, mitochondria and nucleus. The glutaredoxin (Grx) isoenzymes, Grx1 and Grx2, are localized to cytosol and mitochondria, respectively where they participate in the reduction of GSSG or GSH-mixed disulfides through thiol-disulfide exchanges [46]. The monothiol Grx3, which is devoid of oxidoreductase activity, serves as a redox sensor during signaling induced by reactive oxygen or nitrogen species (ROS/RNS) [47]. The localization and high expression of Grx2 in mouse duodenal enterocytes [48] suggest a role in redox reactions. Glutathione peroxidase 1 (Gpx1) and the intestinal specific Gpx2 (formerly known as GSHpx-GI [49]) are the major hydrogen peroxide (H_2O_2) reducing intestinal selenoproteins [50] with a kinetic rate of $\sim 5 \times 10^7\ M^{-1}\ s^{-1}$. Gpx1 exhibits uniform crypt-to-villus distribution while Gpx2 predominates in the crypt region [50,51], and is highly expressed in the ileum and cecum [52]. Interestingly, Gpx1 and Gpx2 are differentially sensitive to selenium (Se) deprivation, the former being more vulnerable to Se deficiency [53], underscoring its importance in intestinal cell survival. The targeting of Gpx4, which catabolizes phospholipid hydroperoxides, to the cytosol and nucleus of human small intestine and colon maybe physiologically meaningful in antioxidant protection [54]. An extracellular Gpx3 isoform functions in mucosal cytoprotection against luminal oxidants [55]. GSH S-transferase (GST) catalyzes GSH-dependent detoxication of luminal electrophiles and carcinogens, and GST expression levels are often used as an index of intestinal tumorigenic potential. The abundance and varied expression of intestinal GST is sensitive to luminal diet and drug exposure [56]. Among the human GST multigenic family of isoenzymes [57], GST Pi and Mu are highly expressed in the small intestine with lower abundance in the colon [56], consistent with rare neoplastic occurrences in the small intestine [58]. The decrease in proximal-to-distal GST in the colon [56] suggests reduced colonic xenobiotic detoxication and increased cancer risk.

2.2.2. Thioredoxin and redox proteins

Intestinal thioredoxin (Trx) proteins function in antioxidant defense and redox regulation [48] through reduction of cysteine disulfides within the Cys-XX-Cys (CGPC) motif in proteins. Trx1 and Trx2 are independently regulated isoforms that are specific to cytosol/nucleus and mitochondria [59]. The E_h of Trx1 and Trx2 are highly reduced, between −300 mV and −330 mV [37]. Accompanying the subcellular distribution of Trx are the seleno-containing Trx reductase isoenzymes that catalyze NADPH-dependent reduction of oxidized Trx. The selenium-Cys residue in the C-terminal motif of the enzyme is essential for catalytic activity. Gut immune response and innate immunity is a recognized role for intestinal Trx. The mucosa expresses high levels of Trx [60] which participates in the antimicrobial function of human β-defensin 1 (hBD-1) [61]. High Trx expression in unstimulated and stimulated lamina propria T lymphocytes (LP-T) reportedly contributes to the maintenance of intracellular redox homeostasis or proinflammatory responses, respectively [62].

Peroxiredoxins (Prxs) are a class of poorly understood redox proteins in the intestine. Prxs are known to be highly expressed in cells (1% of total proteins) with high catalytic rates ($\sim 10^7\ M^{-1}\ s^{-1}$) in H_2O_2 reduction [63], a function that is shared with GPxs. The all-essential peroxidatic Cys participates in H_2O_2-catalyzed formation of Cys-sulfenic acid (Cys-SOH) and disulfide bond with a C-terminal Cys residue, a reversible mechanism implicated in redox signaling [64]. Specific inactivation of membrane-associated Prx1 suggests a novel mechanism for H_2O_2 accumulation and signal propagation initiated at the receptor site [65]. The biological roles for all the known Prx isoforms (Prx 1–6) [66], including in the intestine are incompletely characterized. The finding that Prx exhibits specific subcellular localizations, namely Prx1, 2 in the cytosol, Prx4 in the extracellular space, and Prx3 within the mitochondria suggests specific metabolic functions within these respective compartments [66].

2.3. Homeostasis of luminal and extracellular thiol redox status

2.3.1. Source and function of luminal GSH

Luminal GSH, derived from dietary intake and biliary output [27,67], is a dynamic pool that is important in absorptive and detoxication functions and mucus protection [68]. It is estimated that biliary GSH in duodenal lumen accounted for 50% of hepatic GSH [27,67]. Dietary contribution is more varied, and depends on the consumption of GSH-rich or GSH-deficient foods [69]. Significant roles for luminal GSH includes reduction of dietary disulfides [70], conjugation of electrophiles/xenobiotics [71], scavenging of divalent metals [72], and maintenance of mucus fluidity through assembly/disassembly of mucin oligomers [73]. Moreover, elevated luminal GSH was shown to promote luminal lipid peroxide uptake and reduce lymphatic peroxide transport [29,74]. Apart from mucosal uptake, luminal GSH can be hydrolyzed by the apical membrane γ-glutamyl-transpeptidase [75]. In rat small intestine, substantial GSH hydrolysis occurred preferentially at submillimolar luminal levels [33], the reason yet unknown.

2.3.2. Cysteine redox couple and extracellular thiol/disulfide balance

The extracellular/luminal redox environment is largely determined by the Cys/CySS redox couple, with contributions from the GSH system [70]. The plasma Cys/CySS and GSH/GSSG redox couples are displaced from equilibrium with E_h values tightly regulated at −80 mV and −140 mV, respectively [24,76]. The actual extracellular Cys and CySS concentrations are low, 40 μM and 8–10 μM, respectively, and are modulated by dietary Cys/CySS [77], GSH hydrolysis [78], thiol-disulfide exchange reactions [79], and the Cys/CySS shuttle [80]. An oxidized plasma Cys/CySS redox state has been shown to be associated with vascular pathologies like diabetes, cardiovascular disease, and atherosclerosis [76]; thus, plasma Cys/CySS changes could be predictive of health or disease [81]. Luminal Cys/CySS contributes majorly to maintaining the thiol-disulfide redox state of extracellular proteins [82] and the lumen [68]. In rat intestine, ∼40% of luminal Cys was from GSH hydrolysis which participated in nutrient absorption [83] and mucus preservation [84]. Luminal thiol-disulfide redox status is regulated through the Cys/CySS shuttle [70] involving luminal Cys

export [68], GSSG reduction and CySS formation [70], followed by CySS uptake [85], intracellular GSH-mediated CySS reduction, and Cys re-release into the lumen (Fig. 2). In polarized CaCo-2 cells, E_h for Cys/CySS at the basal and apical surfaces are regulated at different rates [86], implying independent redox signaling mechanisms at opposite polar membrane surfaces.

2.4. Role of gut microbiota

A complex intestinal luminal microflora is represented by 500–1000 species of bacteria [87], and a reducing environment supports a microflora of 100–1000 times greater anaerobes to aerobes in adult gut lumen [88]. The gut microbiota prevents pathogen colonization, supports intestinal nutrition and regulates the mucosal immune system [89]. An over abundance of pathogenic species, a "dysbiotic" flora, contributes to aberrant mucosal immune response and chronic intestinal inflammation [90]. The redox biology of bacteria-intestinal host interaction is incompletely understood. Significantly, gut bacteria produces millimolar levels of hydrogen sulfide (H_2S) [91]; however, high catabolism [92] maintains low luminal H_2S shown to avert the inhibition of mitochondrial cytochrome oxidase [93], ROS production, GSH redox imbalance, and tissue oxidative stress. In colonic HT-29 cells, mitochondrial respiratory rate was actually stimulated by mitochondrial sulfide quinone reductase (SQR)-catalyzed formation of sulfide, an oxidation product of H_2S [94]. The reverse electron flow between SQR and complex I yielded NADH [95]. An antioxidant role for H_2S akin to that in neuronal cells [96] is yet to be defined in intestinal cells.

3. Intestinal oxidative stress and gut pathobiology

3.1. Intestinal oxidative stress and cell fate

3.1.1. Redox modulation of cell transition and growth

Phenotypic transitions of normal intestinal cells from proliferation to differentiation or apoptosis are associated with increasing oxidation of the E_h of intracellular GSH/GSSG or extracellular Cys/CySS redox systems [22,25] (Fig. 3). Significantly, cell transition is unrelated to the E_h of the Trx/TrxS couple [97]. The E_h of intracellular GSH/GSSG varies between −260 mV and −240 mV at proliferation, −220 mV and −200 mV at differentiation, and −170 mV and −150 mV at apoptosis [98]. In neuronal PC-12 cells, exit from quiescence and entry into proliferative, growth arrested, or apoptotic states depended on the severity of the GSH/GSSG imbalance [99]. As for extracellular Cys/CySS, E_h between −80 mV and −50 mV was associated with cell transition from a proliferative to a non-dividing differentiated state [100]. Moreover, the proliferative potential in CaCo-2 cells was modulated by the extracellular Cys-to-CySS ratio [74,99–101]. Since the GSH and Cys redox systems are displaced from equilibrium, possible synergy between the two couples in controlling cell fate remains an unresolved issue. Notably, CaCo-2 proliferative response to changes in Cys/CySS status occurred independently of cellular GSH or GSH synthesis [102], and extracellular Cys oxidation can induce CaCo-2 proliferation through redox activation of growth receptors without altering cellular GSH [100]. Moreover, Cys/CySS (E_h, −150 mV) was able to increase EGFR phosphorylation, p44/42 MAPK signaling and cell growth [103].

Lipid hydroperoxides have been shown to elicit CaCo-2 proliferation at low levels and apoptosis at high levels in association with GSH/GSSG disruption [104]. This means that a change in phenotypic outcome can readily be modulated by an imposed transient [74,99] or sustained [104,105] shift in the GSH/GSSG redox potential such as would occur during acute or chronic intestinal

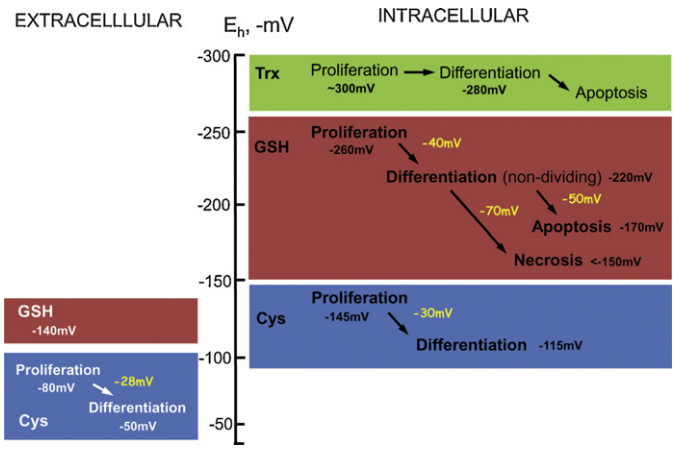

Fig. 3. Association of GSH/GSSG, Cys/CySS and Trx/TrSS redox potential (E_h) with normal intestinal phenotypic transitions. Under physiological conditions, intestinal cell transition from proliferation to differentiation or apoptosis is associated with quantitative changes in the E_h of the GSH/GSSG or Cys/CySS redox couples. A 40 mV oxidation in intracellular GSH/GSSG E_h elicits cell transition from a proliferative to differentiated, non-dividing state. The apoptotic or necrotic states result from an additional 50 mV or 70 mV oxidation. Additionally, intestinal cell progression from proliferation to differentiation is accompanied by ~30 mV oxidation of the E_h of extracellular/intracellular Cys/CySS redox couple. Cell transition is unrelated to the E_h of the Trx/TrxS redox couple. A role for extracellular GSH/GSSG in intestinal phenotypic change is unknown.

oxidative stress. In rat small intestine, normal epithelial growth and apoptosis subscribed to a circadian rhythm corresponding to the animal's feeding and post-prandial periods [106,107]. Sustained consumption of a peroxidized lipid diet disrupted mucosal GSH/GSSG status and caused mucosal cytostasis, while GSH supplementation restored normal mucosal GSH levels and turnover activity [108,109]. Surprisingly, mucosal growth following massive small bowel resection was associated with GSH depletion and an oxidized GSH/GSSG status [110]. This increased proliferative activity may, in part be mediated by elevated plasma Cys/CySS [110], consistent with origination of redox-dependent-growth signals from the basal membrane (Fig. 3).

3.1.2. Mitochondrial GSH, oxidative DNA damage and cell apoptosis

The mitochondrion is an oxidant-prone compartment. The mitochondrial genome, comprising a circular double-stranded DNA organized in nucleoids and lacking histones, is vulnerable to oxidative damage [111]. Early studies demonstrated that the depletion of mitochondrial GSH (mtGSH) sensitized cells to oxidant-induced cell injury [112] and apoptosis [113–115]. Loss of mtGSH was shown to induce transition pore opening [116], inhibition of respiratory complexes [117], decreased ATP [118], and increased ROS production [119]. A proposed vicious cycle of ROS-mtDNA damage through disrupted transcription of electron transport proteins, exaggerated ROS production, and decreased mtGSH also contributed to mitochondrial failure and apoptotic initiation [120]. In colonic HT-29 cells, an added perturbation of cellular NAD^+/NADH and $NADP^+$/NADPH redox status further compromised mitochondrial respiratory function and attenuated cellular NADPH availability [121].

Matrix GSH homeostasis is controlled by cytosol-to-matrix GSH transport [42], dicarboxylate (DIC) and oxoglutarate (OGC) carrier function [42,117,122], and mitochondrial substrates and bioenergetics [123]. MtGSH is a major player in colonic cell survival, and its preservation is critical to mtDNA integrity [124]. In colonic HT-29 cells, mtDNA damage induced by the redox cycling agent, menadione (MQ) was preceded by mtGSH imbalance [115]. The findings that increased or decreased mtGSH import, respectively,

attenuated or exacerbated MQ-mediated oxidative mtDNA damage [115] support the hypothesis that mtDNA vulnerability to oxidative stress corresponded to the mtGSH status. Whether mtGSH quenches ROS or promotes mtDNA repair or both remains an open question.

3.2. Oxidative stress, altered redox status and intestinal pathology

Excessive inflammatory cell-induced ROS generation and tissue oxidative stress are central to the onset and development of chronic gut inflammation. Elevated tissue GSSG [125,126] and/or decreased GSH synthesis [127] have been correlated with the severity of mucosal inflammation or diminished mucosal GSH contents in IBD (intestinal bowel disease) patients [125]. It remains unclear whether intestinal oxidative stress is secondary to the inflammatory process or vice versa. Our finding that impaired mucosal GSH/GSSG preceded the onset of colonic inflammation and clinical colitis in the $CD^{4+}CD45RB^{high}$ T-lymphocyte-reconstituted SCID mouse model of ulcerative colitis is consistent with redox-dependent mechanisms in disease development (Aw, unpublished data). Decreased disease manifestations by N-acetylcysteine support this contention [128,129]. However, the jury is still out on the causal role of mucosal GSH/GSSG disruption in IBD pathogenesis since antioxidant therapies with vitamin C, E, and GSH were without effect in the HLA-B27 transgenic rat model of IBD [130].

Redox signaling in gut inflammation is complex and poorly understood. Attenuated lamina propria T-lymphocyte (LP-T) proliferation is associated with low intracellular GSH [131] and hyporeactivity to luminal microflora [132]. A reduced lamina propria redox milieu can signal LP-T activation [133], accomplished through recruitment of cysteine-secreting blood-borne macrophages that elevated LP-T GSH levels [131]. The perpetuation of an inflammatory phenotype within the lamina propria was thus initiated and sustained by T cell transition from a bacteria-tolerant to a reactive state [134]. Indeed, $CD14^{+}$, cysteine positive macrophages and $CD3^{+}$ LP-T cells with increased cellular GSH are associated with the IBD gut [131].

NF-κB activation can be attenuated by the constitutively high LP-T Trx1 status [62]. Moreover, p65 nuclear translocation was shown to be prevented by bacteria-induced NADPH oxidase-derived H_2O_2 via oxidation of the redox-active Cys of Ubc12, an ubiquitin-like conjugating enzyme [135]. Bacteria-derived ROS can also transiently alter the cytosolic and mitochondrial Trx status and inhibit NF-κB activity [136]. Interestingly, intestinal susceptibility or resistance to luminal pathogens is influenced by the bacterial composition. The vulnerability of C3H/HeOuJ susceptible mice to *Citrobacter rodentium*-induced colitis was prevented by transfer of an enriched Bacteroides microbiota from C57BL/6 resistant mice; GSH/GSSG-mediated changes in inflammatory cytokines and systemic pathogen load was suggested to play a protective role [137]. In IBD, the fragile balance among the luminal bacterial microenvironment, mucosal GSH/GSSG status, and epithelial survival were notably disrupted by increased pathogenic bacterial strains [138], suggesting that probiotic bacteria intervention could enhance epithelial GSH and attenuate mucosal inflammation [139].

A persistent state of tissue oxidative stress is a common link between chronic gut inflammation and increased cancer incidence. Modulation of GSH- and/or Trx-dependent functions were shown to enhance cancer cell proliferation, migration, metastasis, and apoptosis evasion [140,141]. Elevated Trx1 was associated with aggressive growth of primary colorectal cancer cells [142], and is characteristic of colon cancer [143]. Consistent with Grx3 function in tumor growth and survival, protein expression levels were increased ~50-fold [144], and the knock down of Grx3 attenuated NF-κB survival signaling and inhibited tumor progression [145]. Hence, the targeting of intestinal Grx3 expression and NF-κB signaling could underpin colon cancer therapy. The finding that expression of intestinal specific Gpx2 was linked to cancer cell proliferation and early tumor growth supports its role in cancer biology [50]. Colonic susceptibility to oxidative stress and increased cancer risk is further supported by reduced or aberrant Gpx1, Gpx3, and selenoprotein P expressions [146]. Elucidating the role of Gpxs in intestinal inflammation and cancer pathogenesis has been advanced by the creation of genetic mouse model deficient in Gpx genes. Interestingly, mice containing *Gpx2* gene deficiency exhibited minimal gut pathological [147] or IBD [49] symptoms. A compensatory increase in Gpx1 could explain this lack of pathology [148]. However, the Gpx1/2 double knockout mice exhibited severe ileocolitis and distal ileal inflammation [147], consistent with Gpx relevance in gut cancer biology.

4. Concluding remarks and perspective

Loss of intestinal homeostasis is underpinned by mucosal oxidative stress and associated tissue redox imbalance. We now know that homeostatic control of the intestinal epithelial redox environment is central to the functions of the organ in nutrient digestion and absorption, stem cell proliferation, apical enterocyte apoptosis, and immune response. However, the redox mechanisms controlling the fundamental processes of crypt cell signaling and genesis or bacteria-host immunological responses that are critical to intestinal regeneration or survival within the defined microenvironment of the gut are as yet ill-defined. Recent advances in our understanding of intestinal redox biology supports a novel conceptual view that distinct distribution of individual GSH/GSSG, Trx/TrxSS and Cys/CySS redox systems within subcellular organelles can function as unique independent redox control nodes of metabolic pathways. This means that during oxidative stress, a disrupted control node would result in altered protein function through oxidation of catalytic site redox active cysteines. The precise mechanism of how redox-mediated dysregulation of the function of a single protein or protein sets collectively signals mucosal inflammation or aberrant cell turnover are unanswered questions of relevance to intestinal degenerative disorders, such as IBD and cancer.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing the paper.

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