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Polyamines and Gut Mucosal Homeostasis

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Abstract

The epithelium of gastrointestinal (GI) mucosa has the most rapid turnover rate of any tissue in the body and its integrity is preserved through the dynamic balance between cell migration, proliferation, growth arrest and apoptosis. To maintain tissue homeostasis of the GI mucosa, the rates of epithelial cell division and apoptosis must be highly regulated by various extracellular and intracellular factors including cellular polyamines. Natural polyamines spermidine, spermine and their precursor putrescine, are organic cations in eukaryotic cells and are implicated in the control of multiple signaling pathways and distinct cellular functions. Normal intestinal epithelial growth depends on the available supply of polyamines to the dividing cells in the crypts, and polyamines also regulate intestinal epithelial cell (IEC) apoptosis. Although the specific molecular processes controlled by polyamines remains to be fully defined, increasing evidence indicates that polyamines regulate intestinal epithelial integrity by modulating the expression of various growth-related genes. In this review, we will extrapolate the current state of scientific knowledge regarding the roles of polyamines in gut mucosal homeostasis and highlight progress in cellular and molecular mechanisms of polyamines and their potential clinical applications.

Keywords

Ornithine decarboxylase; mucosal injury; restitution; apoptosis; cell proliferation; RNA-binding proteins; microRNAs

Introduction

The gut is an important organ responsible for digestion, absorption, and metabolism of dietary nutrients. The mucosa of the gastrointestinal (GI) tract is lined with epithelium that has the shortest turnover rate of any tissue in the body [1-3]. Maintenance of GI epithelial homeostasis depends on a complex interplay between processes involving intestinal epithelial cell (IEC) proliferation, differentiation, migration, and apoptosis [3-6]. Under normal physiological situations, undifferentiated epithelial cells continuously replicate in the

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proliferative zone within the crypts and differentiate as they migrate up towards the luminal surface of the colon and villous tips in the small intestine [7,8]. To maintain a stable number of enterocytes, cell division must be counterbalanced by the process of apoptotic cell death, a fundamental biological process involving selective cell deletion to regulate tissue homeostasis [3,9,10]. Apoptosis occurs in the crypt area, where it maintains a critical balance in cell number between newly divided and surviving cells, and at the luminal surface of the colon and villous tips in the small intestine, where differentiated cells are lost. This rapid dynamic turnover rate of intestinal epithelial cells is highly regulated and critically controlled by numerous factors, including cellular polyamines [11-13].

Natural polyamines, spermidine, spermine, and their precursor putrescine, are ubiquitous biogenic amines of low molecular weight found in abundance intracellularly in nearly all eukaryotic cells. Polyamines are intimately involved in many distinct cellular functions [11,14,15], but their exact roles at the molecular level remains largely unknown. A series of observations from our previous studies [16-19] and others [20-22] have shown that normal intestinal mucosal growth depends on polyamine availability to dividing cells within crypts, and that polyamines, either synthesized endogenously or supplied luminally, are absolutely required for epithelial cell division. Cellular polyamine content increases rapidly when cells are stimulated to grow and divide, whereas inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, decreases cellular polyamines and represses IEC proliferation both *in vivo* and *in vitro* [13,23,24]. Because of the absolute requirement for cellular polyamines for GI mucosal cell growth and proliferation, the polyamine metabolic pathway has been an attractive therapeutic target for antineoplastic intervention (25). It has also been shown that the excessive expression of ODC and the resulting heightened levels of polyamine in human colon and other cancers (11,15,26) suggests that this sustained overexpression of ODC may lead to malignant transformation (27,28). Since there are already several excellent reviews which focus on polyamines and GI cancers (11,15,29-32), we decided to highlight primarily on the role of polyamines during normal GI mucosal integrity. In this review we discuss what has been defined thus far in regards to the role of polyamines and its clinical significance in gut mucosal homeostasis including mucosal repair, barrier function, growth, and apoptosis.

Polyamine Biosynthesis

Biosynthesis and catabolism of polyamines are carefully controlled processes in all types of eukaryotic cells. Intracellular polyamine levels is maintained and highly regulated by endogenous biosynthesis, exogenous transport, and degradation [11,14,33,34]. Natural polyamines found in the intestinal lumen arise from diet (such as red meat and cheeses), bacteria, and villous extrusion from sloughed epithelial cells. Polyamine metabolism involves both the forward and reverse component pathways, although cellular control of regulatory enzymes and the polyamine transporter act in concert with each other in order to maintain appropriate levels of individual polyamines. Polyamines derive from two amino acids, ornithine and methionine [14,23]. During polyamine synthesis in mammals, ODC, the catalyst for the first rate-limiting step of polyamine synthesis, decarboxylates the amino acid ornithine to form putrescine; propylamine groups are then added to one or both amino groups of putrescine to form spermidine and spermine [12,35,36]. Methionine is the

precursor for S-adenosylmethionine (AdoMet). The decarboxylation product of AdoMet is the precursor of the aminopropyl moieties of spermidine and spermine (Fig. 1).

In contrast, polyamines are degraded by diamine oxidase and spermidine/spermine-N-acetyltransferase (SSAT). Putrescine, spermidine and spermine are interconverted according to cellular physiological needs [36,37]. Polyamine homeostasis is regulated by a feedback mechanism controlled primarily by polyamines themselves via regulation of *de novo* synthesis, release, uptake and catabolism. In mammalian cells, the degradation of ODC is facilitated by a specific ODC-antizyme [38,39], a protein that also appears to downregulate polyamine transport. DL-α-difluoromethylornithine (DFMO) is an irreversible inactivator of the ODC enzyme that specifically inhibits ODC activity. The discovery of DFMO has provided an enormous stimulus to the field of mammalian polyamine biology leading to the unraveling of various polyamine-related mechanisms over the two decades.

Polyamines and Gut Mucosal Repair: Epithelial Restitution and Chronic Healing

Cellular decisions which regulate signaling pathways and control gene expression involved in migration, proliferation, and apoptosis, are required for the successful repair of mucosal damage and wounds [40-42]. The repair of damaged mucosa occurs through two distinct mechanisms: restitution and chronic healing. Mucosal restitution is an important and primary repair modality in the GI tract, and its dysregulation underlies various critical pathological states such as mucosal bleeding and ulcers, disruption of epithelial integrity, and barrier dysfunction [8,43,44]. Epithelial restitution occurs as a consequence of IEC migration to reseal superficial wounds, a process independent of cell proliferation [13,45-48]. In contrast, chronic healing is the much slower mechanism of replacing lost cells through DNA synthesis and cell division, which takes a much longer time compared with epithelial restitution [42,49].

Studies from our own [13,46,50-53] and others [43,44,54,55] have demonstrated a strong relationship between cellular polyamines and mucosal repair processes both *in vivo* and *in vitro*. In a rat stress ulcer model, results show that ODC activity levels were significantly elevated in GI tissues after stress [8,13], and are associated with increased levels of mucosal putrescine, spermidine, and spermine content [8,13]. In contrast, administration of DFMO prevented this normal GI mucosal healing process from occurring [13]. Gastric administration of polyamines immediately following the period of stress prevented the inhibition of repair caused by DFMO and instead increased the normal rate of healing. In order to study the involvement of polyamines in mucosal restitution, an *in vitro* model of healing was developed to demonstrate cell migration after wounding [45,56-59]. In this model, a confluent monolayer of IEC cells were wounded by scraping with a razor blade and cell migration was assessed on short time (~ 6h after wounding) by counting the number of cells that crossed the wounded edge. Cell migration in this model was independent of DNA synthesis but dependent on cytoskeletal reorganization. Several studies showed that cells grown in the presence of DFMO inhibited migration by ~80%, and that exogenous polyamines prevented the decreased migration [45,47,57]. We have demonstrated that polyamines regulate IEC migration by altering K^+ channel activity, membrane potential

(E_m), and cytosolic free Ca^{2+} concentration $[Ca^{2+}]_{cyt}$, and that the induced changes in $[Ca^{2+}]_{cyt}$ exerts its regulatory effects on cell motility through interacting with specific targets during restitution [47,51-53,60,61]. Decreased $[Ca^{2+}]_{cyt}$ by depolarization of E_m inhibited normal cell migration and prevented the restoration of cell migration by exogenous spermidine in polyamine-deficient cells. In contrast, increased $[Ca^{2+}]_{cyt}$ by Ca^{2+} ionophore ionomycin stimulated cell migration in the absence of cellular polyamines [53,57], indicating that polyamine-mediated IEC migration is due partially to increased levels of Kv channel activity.

In another set of experiments we have demonstrated that differentiated IEC-Cdx2L1 cells migrate over the wounded edge much faster than undifferentiated parental IEC-6 cells [45,47,62]. Differentiated IEC-Cdx2L1 cells express higher basal levels of Kv1.1 and Kv1.5 mRNAs and proteins, and depletion of intracellular polyamines decreases the expression of both Kv1.1 and Kv1.5 channel genes, resulting in an inhibition of whole cell K^+ currents, E_m, and reduced resting $[Ca^{2+}]_{cyt}$. The migration rates in differentiated IEC-Cdx2L1 cells are ~4 times that of parental IEC-6 cells [45]. Inhibition of Kv channel expression by depletion of cellular polyamines reduced $[Ca^{2+}]_{cyt}$, resulting in cellular reorganization of cytoskeletal proteins, along with a marked reduction in actomyosin stress fiber formation and inhibited epithelial cell migration. To determine the mechanism by which polyamine-modulated Ca^{2+} induces cell migration during restitution, we have further showed that migration of IECs after wounding is associated with a significant increase in β-catenin tyrosine phosphorylation [56]. Decreased levels of cellular polyamines by DFMO prevented the induction of β-catenin phosphorylation and consequently decreased cell migration after wounding [56], while elevation of $[Ca^{2+}]_{cyt}$ restored β-catenin phosphorylation and stimulated migration in polyamine-deficient cells. These data indicate that β-catenin tyrosine phosphorylation plays a critical role in polyamine-dependent cell migration and that polyamines induce β-catenin phosphorylation at least partially through $[Ca^{2+}]_{cyt.}$

During restitution, intestinal columnar cells must undergo rearrangement –flattening, stretching, migrating, and the eventual repolarizing of their cytosol and membrane [44,45,63,64]. Major stimuli and modulators of epithelial cell migration comes from local growth factors produced at the site of injury and elsewhere (e.g. platelets and macrophages) as well as from metabolic substrates, the extracellular matrix, regulatory peptides, integrins, cytokines, and polyamines. These growth factors stimulate cytoplasmic and cytoskeletal rearrangement in order for migration to occur [65,66]. More specifically, genes affecting the components that make up these structures, including microfilaments and stress fibers composed of actin and/or myosin, are key to successful migration and restitution. Several studies have indicated that small GTP-binding proteins such as RhoA, Rac1, and Cdc42 play an important role in polyamine-dependent IEC migration after wounding. We have shown that polyamines are required for expression of Rac1 protein in differentiated IEC-Cdx2L1 cells and are implicated in modulating the interaction between Rac1 and phospholipase C-γ1 (PLC-γ1) after wounding, partially through $[Ca^{2+}]$cyt. Fig. 2 shows that the depletion of cellular polyamines decreased the formation of Rac1/PLC-γ1 complexes, attenuated store depletion-induced Ca^{2+} influx, and repressed cell migration after wounding. We also reported that PLC-γ1 expression requires polyamines and that polyamine-induced PLC-γ1 is

involved in the control of $[Ca^{2+}]_{cyt}$ during epithelial restitution [47]. These results prove the possibility that Rac1/PLC-γ1 complexes are necessary for the stimulatory effect of polyamines on Ca^{2+} influx after wounding. Studies from these experiments suggest that Rac1 functions as an upstream regulator of PLC-γ1-induced Ca^{2+} signaling and that induced interaction between Rac1 and PLC-γ1 enhances polyamine-dependent cell migration after wounding by increasing $[Ca^{2+}]_{cyt}$. Given the fact that Rac1/PLC-γ1-induced Ca^{2+} signaling is highly regulated by cellular polyamines and that levels of tissue polyamines in the damaged intestinal mucosa are dramatically increased [51,67], activation of this signaling pathway is crucial for polyamine-dependent IEC migration after injury and contributes to the maintenance of intestinal epithelial homeostasis. Our recent studies further revealed that the major store-operated Ca^{2+} (SOC) channels such as Transient Receptor Potential Channel 1 (TRPC1), TRPC5, and Stromal Interaction Molecule 1 (STIM1) are upstream of Rac1/PLC-γ1 signaling and plays a critical role in IEC migration after wounding [52,68].

Deeper mucosal defects such as ulcers, often a result of tissue necrosis and penetration of the muscularis mucosa, and chronic injury, necessitate a somewhat different repair process. As opposed to the rapid mucosal restitution that occurs in the acute phase of GI mucosal injury, chronic healing process requires much more in the way of cellular replication, protein synthesis and *de novo* DNA and mRNA synthesis [49]. Because several studies indicate that cellular polyamines are required for the growth of all eukaryotic cells, it is not surprising that the cell replacement stage of mucosal wound healing is dependent on polyamine levels. It has been shown that there was almost no replacement of gastric mucosal cells 24 h after stress in rats treated with DFMO [43]. Polyamine depletion blocked the increases in protein, RNA and DNA synthesis, and content that normally follow damage [69]. One of the earliest events that are triggered in the stress ulcer model is a significant transient increase in the expression of the c-*fos* and c-*myc* protein levels that were preceded by increases in ODC activity and putrescine levels [46,70,71]. Blocking ODC with DFMO totally prevented the increased expression of protooncogenes. *In vitro* wound healing model results also show that the expression of c-*fos*, c-*myc*, and c-*jun*, protooncogenes is required for healing, which was inhibited by polyamine depletion. These cellular protooncogenes are responsible for the regulation of the cell cycle and are involved in healing as well as normal growth and development [72]. Thus polyamines are essential for healing process by enhancing expression of these protooncogenes [71,72]. Several studies demonstrated that cellular polyamines plays a critical role during chronic healing by altering signaling mechanisms involving Wnt, TGF-β, MAPK, ERK1/2, EGF, and EGF-R [49,73-75].

Polyamines and Gut Epithelial Barrier Function

Epithelial cells line the intestinal mucosa and form an important barrier that protects the subepithelial tissue against a wide array of noxious substances, allergens, and luminal microbial pathogens [2,3]. The effectiveness and stability of this epithelial barrier depends on the activity of junctional complexes that include tight junctions (TJs), adherens junctions, desmosomes, and gap junctions, all of which seals epithelial cells together in a way that prevents even small molecules from leaking between cells [76-78]. An increasing body of evidence indicates that formation of adherens junctions is essential for the assembly of TJs between epithelial cells and that alteration in levels of the cadherin-dependent adherens

junctions regulates the stability of TJ complexes and affects intestinal epithelial paracellular permeability [79-81]. We [82-84] have demonstrated that polyamines regulate the intestinal epithelial barrier function and that polyamine depletion increases epithelial paracellular permeability partially by repressing expression of Zona Occludens-1 (ZO-1), occludin and E-cadherin. Our studies have further shown that polyamines modulate expression of various intercellular junction proteins through distinct cellular signaling pathways. In this regard, polyamines promote E-cadherin mRNA translation and increases its protein stability [85], whereas polyamines modulate ZO-1 transcription by altering the interaction of the ZO-1 gene promoter [86]. We also provide further evidence showing that polyamines are necessary for E-cadherin transcription by activating c-Myc interaction with the E-Pal box in the proximal region of the E-cadherin promoter [85].

Occludin is a transmembrane TJ protein that plays an important role in TJ assembly and regulation of the epithelial barrier function. Our most recent studies indicated that decreasing cellular polyamines inhibited occludin expression and also decreased the levels of phosphorylated Human Antigen R (HuR) [84]. The RNA-binding protein HuR modulates the stability and translation of many target mRNAs leading us to investigate whether polyamines regulate occludin expression via HuR and subsequently the intestinal epithelial barrier function. Although the mechanisms by which polyamines regulate occludin expression remains to be fully elucidated, our studies have shown that polyamine depletion by DFMO decreased HuR/occludin mRNA complexes and repressed occludin mRNA translation. Exogenous polyamine putrescine, when given together with DFMO, prevented Chk2 kinase inhibition and restored HuR phosphorylation and its binding affinity to occludin mRNA, thereby promoting occludin translation. Consistent with our current findings, polyamines are also shown to increase HuR's binding to c-Myc mRNA through enhancement of Chk2-dependent HuR phosphorylation in IECs [84,87]. *In vivo* studies from our laboratory further revealed that Chk2-dependent HuR phosphorylation is implicated in the maintenance and re-establishment of gut barrier integrity under critical surgical stress. CLP stress decreased Chk2 levels, reduced HuR/occludin mRNA complexes, and inhibited occludin abundance, thus contributing to the pathogenesis of gut barrier dysfunction [84]. In contrast, induced Chk2-dependent HuR phosphorylation appears to be crucial for the recovery of occludin expression and gut barrier function. These results indicate that polyamines are implicated in the Chk2-dependent HuR phosphorylation that regulates occludin mRNA translation and helps to maintain and re-establish epithelial barrier function in the intestinal tract when responding to septic stress [84].

Polyamines and Mucosal Growth

Mucosal cell turnover is extremely fast; in rodent gut, cells are replaced approximately every three days and in humans about every four days [3,88,89]. Several studies show that cellular polyamine content increases rapidly in cells stimulated to grow and divide, while reducing cellular polyamines by inhibiting ODC [11,90-94] represses IEC proliferation *in vivo* and *in vitro* [13,72,95]. Since the recognition that polyamines are absolutely required for mammalian cell growth, the targeting of their function and metabolism has been an attractive strategy for anti proliferative therapy [26,96,97]. Polyamines are shown to positively regulate the transcription of growth-promoting genes such as *c-fos, c-jun, and c-*

myc [70-72] and negatively affect growth inhibiting genes including p53, NDRG1, NPM, JunD, and TGFβ/TGFβ receptor at the posttranscriptional level [94,98-106].

Our recent studies revealed that depletion of cellular polyamines increases the nuclear abundance of ATF-2 by stabilizing its mRNA, which is associated with a decrease in the levels of cyclin-dependent kinase 4 (CDK4) and cell proliferation [107-109]. We have shown a novel function of ATF-2 in the modulation of CDK4 expression and demonstrate that induction of ATF-2 represses CDK4 gene transcription, thus contributing to the inhibition of IEC proliferation following polyamine depletion [107]. Studies aimed at characterizing the molecular aspects of this process indicate that induced ATF-2 in polyamine-deficient cells physically interacts with JunD and forms ATF-2/JunD heterodimers that directly bind to the CDK4-promoter.

Most recently, we went further to provide evidence that polyamines promote the translation of CDK4 by repressing CUG-binding protein 1 (CUGBP1) and microRNA-222 (miR-222) in IECs. Specific inhibition of CDK4 activity delays cell-cycle progression and results in growth arrest in the G1 phase, similar to the phenotype observed in polyamine-deficient IECs [110]. We reported that CUGBP1 and miR-222 jointly bind the CDK4 mRNA and repress CDK4 translation synergistically. First, we determined the effect of cellular polyamines on global miRNA expression by miRNA array analysis. A comparison of the miRNA expression profiles in untreated relative to polyamine-deficient cells revealed several increased miRNAs after polyamine depletion, including miR-222, miR-195, miR-140, and miR-29b (Fig. 3A, top). In contrast, increasing cellular polyamines by the overexpression of *ODC* gene in IEC cells (ODC-IEC) decreased the levels of some miRNAs, such as miR-222 and miR-29b (Figure 3B, top). Although a sizable subset of miRNAs showed altered abundance in IECs after modulating polyamines, we focused on miR-222, based on its strong dependence on polyamine abundance and its predicted interaction with the CDK4 mRNA. Real-time quantitative PCR (Q-PCR) analysis confirmed changes in the levels of miR-222 after altering the levels of cellular polyamines and revealed that miR-222 levels increased by polyamine depletion (Fig. 3A, bottom) but decreased in ODC-IECs, which contained high polyamine levels (Fig. 3B, bottom). Taken together, these findings show that polyamines negatively regulate CUGBP1 and miR-222 expression in normal IECs.

In a second set of experiments, we found that CUGBP1 directly interacts with both the 3′-UTR and coding region (CR) of CDK4 mRNA, but miR-222 only binds to the CDK4 CR. Moreover, polyamine depletion inhibited CDK4 translation by inducing cytoplasmic CUGBP1 and miR-222 levels, whereas increased levels of cellular polyamines stimulated CDK4 expression by decreasing CDK4 mRNA associations with CUGBP1 and miR-222 [110]. Because intracellular polyamines are tightly regulated by stress stimulation and the status of cell growth, this suggests that polyamine-mediated activation of CDK4 expression by targeting CUGBP1 and miR-222 directly regulates the growth of the intestinal mucosa and thereby contributes to maintaining the integrity of the intestinal epithelium.

Polyamines and Apoptosis

It has been shown that apoptosis, rather than simple exfoliation of enterocytes, accounts for the majority of cell loss at the luminal surface of the colon and villous tips in the small intestine [3]. Apoptosis also occurs in the crypt area. Previous work from our lab [19,71,101,104] and others [10,44,55] have demonstrated that polyamines are crucial for the maintenance of epithelial homoeostasis and that depletion of cellular polyamines promotes the resistance of IECs to apoptosis through multiple signaling pathways. Although the exact roles of polyamines in apoptotic pathways has been rather controversial, depending on the cell type and death stimulus, polyamine depletion by inhibition of ODC with DFMO promotes resistance to tumor necrosis factor-α (TNF-α)/cycloheximide (CHX)-induced apoptosis in normal IEC-6 cells. We have also shown that polyamines downregulate NF-κB activity and that depletion of cellular polyamines increases NF-κB transcriptional activity, thus stimulating the expression of c-IAPs [106,111]. Polyamines are also needed for the inhibition of focal adhesion kinase (FAK) [112] and Akt [113], as polyamine depletion induces the phosphorylation of FAK and Akt and increases their kinase activities. Recently, polyamines are shown to inhibit the expression of ATF-2 and XIAP genes at the post-transcriptional level, as decreasing the levels of cellular polyamines increases the steady-state levels of ATF-2 [107,109] and XIAP [111] through stabilization of their mRNAs.

To investigate the physiological consequences of inducing endogenous XIAP by HuR following polyamine depletion, we studied the possible involvement of this process in regulating IEC apoptosis. We first examined spontaneous apoptotic cell death without any challenge of apoptotic stimulators after inhibition of XIAP expression by using siRNA targeting XIAP mRNA (siXIAP) or siHuR in the absence of cellular polyamines. Transient transfection with the siXIAP or siHuR prevented the increased expression of XIAP in polyamine-deficient cells (Fig. 4A) but failed to directly induce apoptosis in polyamine-deficient cells (Fig. 4B, left). Second, we determined whether XIAP silencing altered the polyamine depletion-mediated resistance to apoptosis elicited by treatment with TNF-α/CHX. As shown in Figure 4B*a*, when control cells were exposed to TNF-α/CHX for 4 h, morphological features characteristic of programmed cell death were observed and annexin V staining showed significant phosphatidylserine presence in the cell membrane, a classic indicator of apoptotic cells (Fig. 4B*a*, right). This increased resistance to TNF-α/CHX-induced apoptosis was not altered when polyamine-deficient cells were transfected with C-siRNA (Fig. 4B*b*), but was lost when XIAP expression was silenced by siXIAP (Fig. 4B*c*) or siHuR (Fig. 4B*d*). The percentages of apoptotic cells (Fig. 4C) in DFMO-treated cells transfected with siXIAP or siHuR were significantly increased compared with those observed in DFMO-treated cells transfected with C-siRNA after exposure to TNF-α/CHX. These results indicate that the HuR-mediated increase in XIAP expression contributes to an increase in resistance to apoptosis following polyamine depletion.

Given our long-standing interest in understanding polyamine function in gut mucosal homeostasis, we also analyzed the association of HuR with p53, NPM, AUF-1, ATF-2 and MEK1 [105,107,109,114-116]. Increased levels of cytoplasmic HuR following polyamine depletion is associated with the abundance and steady state levels of p53, ATF-2, and MEK1 mRNA complexes. Taken together, our studies revealed that HuR-mediated protein

expression plays an important role in the regulation of IEC apoptosis and thus is implicated in the maintenance of gut mucosal homeostasis.

Summary and Clinical Significance

Among mammalian cells the link between polyamine metabolism, synthesis, and neoplastic growth has been well established [26,117-121]. The role of polyamines is extensive and differs among various cell types. Several studies have showed that increased polyamine synthesis is linked to colon carcinogenesis in preclinical models and in humans. Dietary polyamines are an important factor in adenoma prevention, whereas controlling exogenous polyamines is an adjunctive strategy to chemoprevention with polyamine-inhibitory agents [11,15,12,23]. DFMO has been in use as a chemotherapeutic agent for many years and polyamines are thought to be potentially useful for enhancing drug absorption [122-125]. In addition, polyamine metabolism is affected by a variety of non-steroidal anti-inflammatory drugs (NSAIDs) [126,127]. Polyamines are strongly reactive within various cell types of the digestive tract including pancreatic acinar cells, gastric chief cells, insulin cells and cells of the submandibular gland [128-130]. Polyamines also function as “important growth factors in breast milk” which have important implications in neonatal gut maturity and potential formula supplementation [123,131,132]. With the wide array of cell types and roles of cellular polyamines, the possibilities for clinical application seem limitless.

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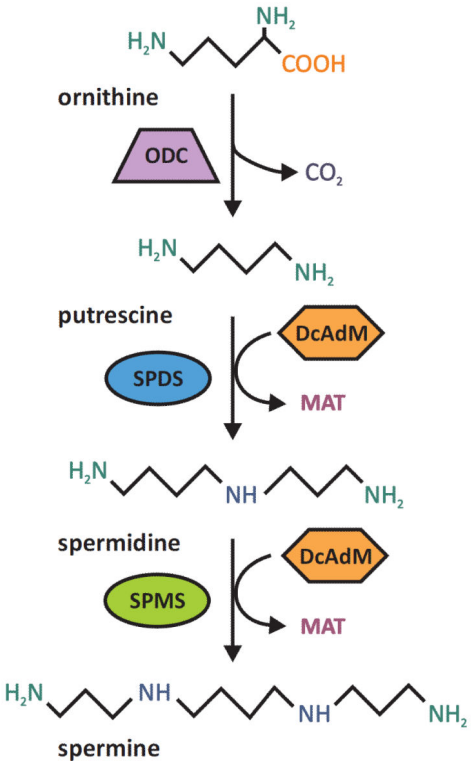

Fig. 1.

Polyamine biosynthesis pathway. ODC – ornithine decarboxylase, SPDS – spermidine synthase, SPMS–spermine synthase, DcAdM–decarboxylated S-adenosyl-L-methionine, MTA-5′-Methylthioadenosine

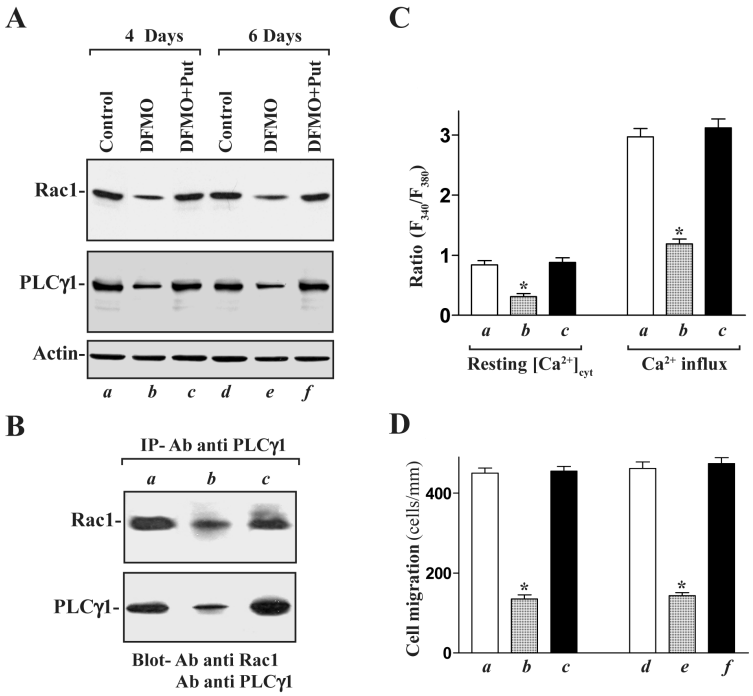

Fig. 2.

Changes in levels of Rac1 and PLC-γ1 proteins, Rac1/ PLC-γ1 complex, $[Ca^{2+}]_{cyt}$, and cell migration in the presence or absence of cellular polyamines. ***A****:* representative immunoblots of Western analysis for Rac1 and PLC-γ1 proteins. Differentiated IEC-Cdx2L1 cells were grown in the DMEM media containing 5 mM α-difluoromethylornithine (DFMO) alone or DFMO plus 10 μM putrescine (Put) for 4 and 6 days. Levels of Rac1 and PLC-γ1 proteins were measured by Western blot analysis. ***B****:* levels of Rac1 and PLC-γ1 proteins in the complex immunoprecipitated (IP) by the anti-PLC-γ1 antibody from cells exposed to DFMO or DFMO + Put for 4 days. ***C:*** summarized data showing resting $[Ca^{2+}]_{cyt}$ concentrations (*left*) and the amplitude of CPA-induced Ca^{2+} influx (*right*) from cells described in ***B***. Values were means ± SE; $n = 20$. *$P < 0.05$ compared with control cells and cells exposed to DFMO plus Put. ***D***: summarized data showing cell migration 6 h after wounding in cells described in ***A***. Values are means ± SE of data from 6 dishes. *$P < 0.05$ compared with control cells or cells treated with DFMO plus Put.

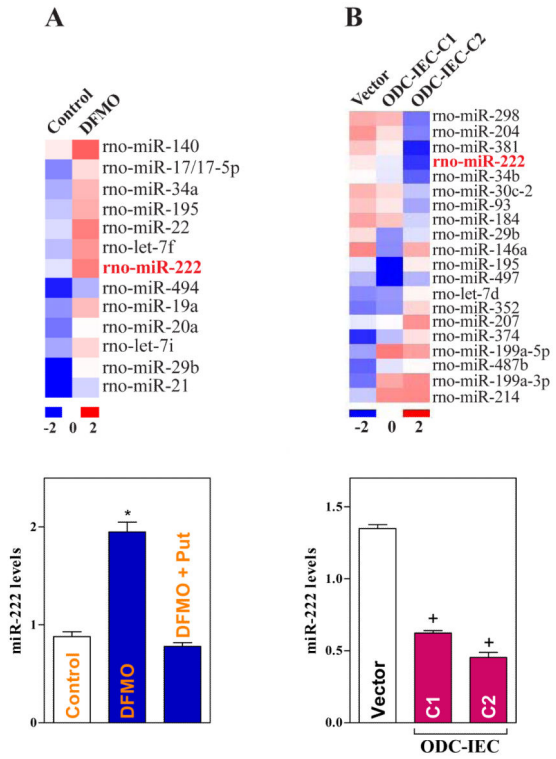

Fig 3.

Changes in microRNA profile and miR-222 expression after altering the levels of cellular polyamines. (A) Changes of global miRNA profile (top) as measured by miRNA array and the levels of miR-222 (bottom) as examined by Q-PCR analysis after polyamine depletion by treatment with DFMO. (B) Changes of global miRNA profile (top) and miR-222 levels (bottom) in ODC-IECs after increasing polyamine levels by ectopic ODC overexpression.

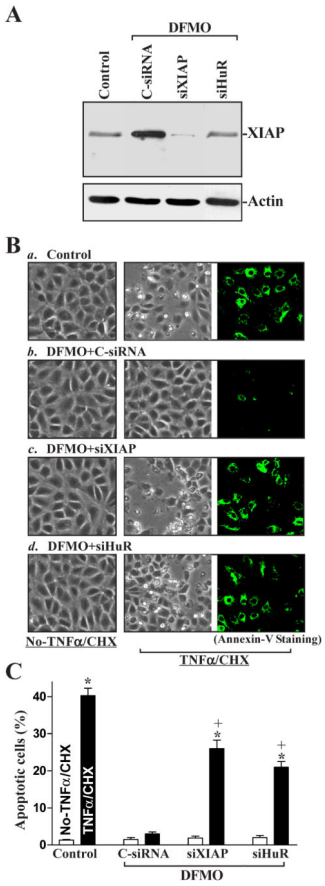

Fig 4.

Effects of XIAP silencing or reduced XIAP by silencing HuR on apoptotic sensitivity in polyamine-deficient cells. (A) Representative immunoblots for XIAP protein. Cells were grown in the cultures containing DFMO for 2 days and then transfected with either siRNA specifically targeting XIAP mRNA CR (siXIAP), siHuR or C-siRNA. The levels of XIAP protein were measured by western blot analysis 48 h after transfection. (B) TNF-α/CHX-induced apoptosis in cells described (A): (a) control cells; (b) DFMO-treated cells transfected with C-siRNA; (c) DFMO-treated cells transfected with siXIAP; and (d) DFMO-treated cells transfected with siHuR. Apoptosis was measured by morphological analysis (middle) and ApoAlert annexin V staining (right) 4 h after treatment with TNF-α/CHX. Original magnification: x150. (C) Percentage of apoptotic cells as described in (B). *P<0.05 compared with no-TNF-α/CHX. +P<0.05 compared with DFMO treated cells that were transfected with C-siRNA and then treated with TNF-α/CHX for 4 h.