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Possible Therapeutics for Pseudomyxoma Peritonei: A Rare, Lethal, and the Least Investigated Disease

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Abstract

Pseudomyxoma peritonei (PMP) refers to a growth disorder characterized by glycoprotein neoplasm in the peritoneum, where mucin oversecretion occurs. The tumors of the appendix region are well associated with PMP; however, ovarian, colon, stomach, pancreas, and urachus tumors have also been linked to PMP. Other mucinous tumors in the pelvis, paracolic gutters, greater omentum, retrohepatic space, and Treitz ligament can be the reason for PMP. Despite being rare and having a slow growth rate, PMP can be lethal without treatment. It is treated with neoadjuvant chemotherapy with the option of cytoreductive surgery and intraperitoneal chemotherapy. In the current study, we hypothesize that there may be novel gentle ways to inhibit or eliminate the mucin. Dr. David Morris has used mucolytics—such as bromelain and N-acetyl cysteine to solubilize mucin. In the present review, we aimed to study the regulation of mucin expression by promoter methylation, and drugs that can inhibit mucin, such as boldine, amiloride, naltrexone, dexamethasone, and retinoid acid receptors antagonist. This review also explored some possible pathways, such as inhibition of Na+, Ca2+ channels and induction of DNA methyltransferase along with inhibition of ten-eleven translocation enzymes, which can be good targets to control mucin. Mucins are strong adhesive molecules that play great roles in clinging to cells or cell to cell. Besides, they have been greatly involved in metastasis and also act as disease markers for cancers. Diagnostic markers may have exclusive roles in disease initiation and progression. Therefore, the present review explores various drugs to control and target mucin in various diseases, specifically cancers.

Keywords

- pseudomyxoma peritonei
- retinoid
- boldine
- therapy
- N-acetyl cysteine

Introduction

The clinical term *pseudomyxoma peritonei* (PMP) refers to a growth disorder characterized by the buildup of grossly visible glycoprotein neoplasm deposits within the peritoneal cavity. The precise explanation for PMP is presently unknown, for no specific genetic or environmental factors have been found to cause it. Varied forms of tumors may

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result in PMP, and it is not well-known why some tumors cause PMP and others do not.[1] Appendix tumors are related to PMP; however, tumors of the ovary, colon, stomach, pancreas, and urachus have also been related with PMP[2,3]

Cells from a glycoprotein tumor (usually of the appendix) unfold into the abdominal cavity and attach the serous membrane to other organs within the abdomen. Then, the tumor cells grow and produce glycoprotein that builds up and contributes to the signs and symptoms observed in people with PMP.

Diseases in the pelvis, paracolic gutters, greater omentum, under the right hemidiaphragm, in the retrohepatic space, and in the Treitz ligament are all characterized by mucinous tumors that may be the reason for PMP.[4] Pseudomyxoma peritonei is a clinical designation for mucinous tumors that have spread beyond a localized focus and across the abdominopelvic cavity. A few number of PMP cases originate in the colon, as well as in other locations, such as the pancreas, urachus, or appendiceal-type mucinous tumors and ovarian teratomas.[5–7]

Despite its slow growth rate, PMP is lethal without treatment. After assessing tumor response, systemic neoadjuvant chemotherapy with the option of cytoreductive surgery and intraperitoneal chemotherapy may be prescribed for patients with high-grade disease.[8] Cytoreductive surgery is an attempt to rid the body of all of the mucin, followed by hyperthermic intraperitoneal chemotherapy (HIPEC) and early postoperative intraperitoneal chemotherapy (EPIC), known as the Sugarbaker technique.[9] It is a brutal operation—dubbed as the mother of all surgeries. Essentially, the surgeons remove the affected non-vital organs, cauterize, laser, and scrape the mucin off the vital organs and then fill the abdominal cavity with chemotherapy drugs for a determined period of time. These treatment methods become brutal for the patients and even more brutal for patients with recurrence. A subset of patients with low-grade disease may remain asymptomatic for prolonged intervals, but most patients who are managed by repeated surgical debulking alone will succumb to intestinal obstruction, terminal starvation, and/or surgical complications. Clinicians have been treating patients with chemotherapy that has been tested in colon cancer, because that is the closest related organ. However, studies show that PMP is quite distinct—closer in molecular landscape to pancreatic neuroendocrine tumors.

There is a major chance of disease recurrence. Such a brutal surgical procedure repeated in case of recurrence can be life-threatening.[10] In case of more common cancers, the metabolic pathways are well known, but with PMP it is less clear. In the current study, we hypothesize that there may better novel ways to eliminate or suppress the mucin. For instance, Dr. David Morris has been using mucolytics—such as bromelain and N-acetyl cysteine (NAC)—to solubilize the mucin. We believe that this procedure, or something similar to this, can be a much gentler way to manage this disease. In the present review, we are interested in the regulation of mucin expression by promoter methylation, several drugs that can inhibit mucin, such as boldine, amiloride, naltrexone, dexamethasone, and retinoid acid receptors (RAR) antagonist. Additionally, some possible targeted pathways can be inhibiton of Na+, Ca2+ channels and induction of DNA methyltransferase (DNMT) along with inhibition of ten-eleven translocation (TET) enzymes. These can prove to be good ways of managing PMP.

Boldine

Boldine is the primary alkaloid in *Peumus boldus Molina*, which has long been used in conventional medicine to treat digestive problems. It is a compound that has been shown to have excellent antioxidant and antiinflammatory effects.

Probable Effect of Boldine on Goblet Cell, Mucin Secretion and Inflammation

Boldine can significantly reduce myeloperoxidase (MPO) activity and glial fibrillary acidic protein (GFAP) as well as decrease immunoreactivity of tumor necrosis factor alpha (TNF-α) and iNOS.[11] To crosscheck boldine, Resendiz-Albor et al.[12] found that treatment with muscarine augmented the number and size of goblet cells along with mucus in the lumen of the small intestine. In their study, an increased activity of MPO in the group treated with muscarine was noticed. Thus, the increase in MPO activity seems to be directly proportional to an increase in the number of goblet cells along with mucin secretion, and, since boldine has an MPO-inhibitory effect, it can be a good solution. If MPO can be one of the pathogenic events in PMP, boldine will be effective. We have not found studies which show the role of MPO in PMP, but, theoretically, this might become useful for the management of PMP; thus, further experiments are encouraged in this direction.

The expression of GFAP in the mucosal plexus is highly increased in the inflamed colon of patients with ulcerative colitis (UC) and infectious colitis, as well as in Crohns disease (CD). All these diseases are associated with overproduction of mucus, which may directly or indirectly involve goblet cells. Additionally, the non-inflamed colon of CD patients has been shown to present a reduced GFAP expression. This suggests that either increased GFAP is responsible for inflammation or vice versa. Glial fibrillary acidic protein can be one of the pathogenic events involved in mucin secretion. Thus, direct studies on this topic are required. If so, GFAP is also inhibited by boldine,[11] which can be quite effective in the treatment of CD, as well as PMP. Walstab et al.[13] have found that boldine and menthol inhibited the 5-HT-induced activation of 5-HT3 receptors in the low and micromolar ranges respectively. The 5-HT_3 receptor is a member of the Cys-loop family of ligand-gated ion channels. Walstab et al.[13] have also found that boldine was a competitive antagonist of both 5-HT3 receptors, being 6.5- to 10-fold more potent toward 5-HT3 A- than 5-HT3 AB receptors. The 5-HT/5-HT_3 receptor and SP/NK_1 receptor pathways play pathogenic roles in colonic inflammation,[14] which is associated with increased mucin secretion and later may give rise PMP.

Boldine Inhibits Oxidative Stress and Immunomodulatory Functions

Treatment with boldine exerts endothelial protective effects in hypertension by lowering peroxynitrite production, inhibiting NADPH mediated superoxide production and down-regulation of p47 (phox) protein expression in the aorta of spontaneously hypertensive rats (SHR).[15,16]

Boldine is also capable of attenuating oxidative stress by decreasing malondialdehyde and nitrite levels in brain and significantly increases glutathione in the brain of both young and aged mice. Boldine, as a suppressor of oxidative stress, can become very useful in case of PMP. Boldine showed no genotoxic activity with or without metabolic activation as tested by an earlier study.[17]

The administration of boldine to dextran sulfate sodium (DSS)-induced mice protected them from colon damage,[18] shown by a reduction in the activity of MPO and CD 68+ expression. Boldine significantly reduced the severity of the inflammation, crypt damage, and infiltration of leukocyte in the mucosa.[18] It significantly decreased the production of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-17, signal transducer and activator of transcription-(p-STAT3) (Y705), and nuclear factor (p65-NF-κB). Data from earlier studies[18] demonstrated that boldine may have selective immunomodulatory (by inhibition of p65-NF-κB and STAT3 signaling pathways) effects, and it can be beneficial in colitis. Infusion of boldine protects from oxidative hepatic damage caused by cisplatin, attributed to natural antioxidants of boldine, specially catechin. These findings suggest the potential use of the boldine as a chemoprotector,[19] which can be quite useful during PMP chemotherapeutic surgery. In a study with rats, Yu et al.[20] found that N-allylsecoboldine relaxes rat's aorta by blocking Ca2+ channels, and that it also has an antagonistic effect on alpha 1-adrenoceptors. Calcium is an important regulator of mucin secretion and goblet cell regulation, which will be discussed later in detail.

Boldine as an Antiproliferative Agent, with Cell-Cycle Arrest and Apoptotic Property

Several studies have suggested that boldine has antioxidant activity, besides possessing apoptotic nature via Bax over-expression, and cleaving caspase 3.[21] Boldine interacts with the peroxisome proliferator-acitvated receptor (PPAR) response element and could modulate PPAR-responsive genes.[22] An in-vitro study[23] showed that boldine reduced the viability and proliferation of Michigan Cancer Foundation (MCF)-7 cells and was cytotoxic to them. It decreased bromodeoxyuridine incorporation and histone H3 phosphorylation, but did not induce apoptosis in this case. In a dose-dependent manner, boldine treatment activated p38, extracellular signal-regulated kinase protein (ERK) and JNK in the mitogen-activated protein kinase pathway.[23] Boldine induced apoptosis in breast cancer cells, as indicated by an increased lactate dehydrogenase, increased membrane permeability, and DNA fragmentation. In addition, boldine induces cell-cycle arrest at G2/M phase. The anticancer mechanism is attributed to disruption of the mitochondrial membrane potential and release of cytochrome c in MDA-MB-231.[24]

Gerhardt et al.[25] found that boldine reduces cell viability and proliferation in T24 cells (bladder cancer cells) by arresting the cell cycle at the G2/M phase and inducing apoptosis. In their study,[25] it inactivated the ERK, and apoptosis wascorrelated with the inactivation of AKT and activation of glycogen synthase kinase3β (GSK–3β) proteins. Boldine can alter the cell cycle, and it causes a G2/M arrest in U138-MG cells with no toxic effect on non-tumor cells at the same concentrations.[26] These results led us to speculate that boldine can become a valuable anticancer agent. It down-regulated Bcl-2 and heat shock protein 70 (HSP-70), and upregulated Bax in the MDA-MB-231 cell line.

Amiloride

Amiloride is used along with other medications to treat high blood pressure, swelling due to heart failure, or liver cirrhosis. It is sold under the trade name (AA Pharma Inc. DATE OF PREPARATION: 1165 Creditstone Road, Unit #1 August 25, 2010 Vaughan, Ontario M9L 1T9). Amiloride is classified as a potassium-sparing diuretic. Despite being used only to control blood pressure and swelling, it has shown other functions as well, which need to be further studied. Amiloride has been found[27] to significantly reduce pulmonary mortality, airway mucus obstruction, epithelial necrosis, goblet-cell metaplasia, and airway inflammation in β-epithelial Na^+ channel (βENaC)-overexpressing mice. Amiloride can reduce goblet-cell differentiation through NHE blocking, since NHE plays an important role in goblet-cell differentiation and function.

Using HT29-MTX cells as an in vitro model, Xu et al.[28] detected that abundant Na^+/H^+ exchanger isoform 8 (NHE8) mRNA are present in goblet cells. Through immunohistochemical staining, they[28] located the NHE8 protein on the plasma membrane and in the intracellular compartments in goblet cells. Furthermore, NHE8 expression in goblet cells is regulated by the proinflammatory cytokine TNF-α. The expression of NHE8 in HT29-MTX cells was significantly reduced at both mRNA and protein levels in the presence of TNF-α.[28] This proves that this Na^+/H^+ exchanger (NHE) is one of the major components of goblet cells, and so goblet cell produces more of it.

Another study[29] showed that rats with triple negative breast cancer (TNBC)-induced colitis had decreased goblet cells and mucin staining, which was directly correlated with the decreased expression of NHE2.[29] These two studies[28,29] make us think that increased NHE expression means increased NHE activity, which is one of the major requirements of a functioning goblet cell. Besides, the second study[29] suggests that NHE is also responsible for goblet-cell production or differentiation. Thus, amiloride is helpful to control goblet cells via its ability to inhibit NHE. Most of the function of this diuretic drug revolves around its ability to block NHE; even if it has any other function to control cancer cells, it all depends on its NHE blocking. Mucin secretion is also a Ca2+-dependent process. However, whether the source of

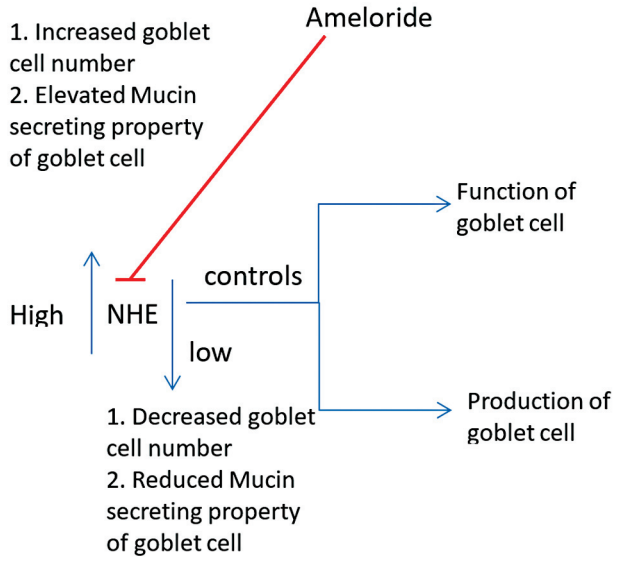

Fig. 1 Amiloride inhibits Na^+/H^+ exchanger.

Ca2+ is intracellular, extracellular, or both is unclear. There is growing evidence of the requirement of extracellular Ca2+ for mucin secretion in different cell types under physiological conditions.[30] Secretion by ATP-mediated pathway depends on extracellular Ca2+. Ca2+ entry (involving transient receptor potential cation channel subfamily M member 5 [TRPM5], as shown in previous studies[31]) from extracellular enviroment, is, however, necessary for both MUC5AC and mucin 2 (MUC2) release. Inhibition of Na entry results in membrane hyperpolarization, which activates Ca2+ entry by dihydropyridine-sensitive calcium channels.[32]

Thus, altogether, amiloride tries to balance the extracellular pH, and it enables cells to create an extracellular lower pH (known to induce oncogenic response). Additionally, if we look into it carefully, it also generates an intracellular ionic stress by not allowing H+ release and allowing Ca2+ entry. Ionic stress is never good for cell survival; thus, it may kill the cells quickly. Therefore, amiloride can be given a thought in order to either lower the goblet cell function or inhibit its production. Since, PMP severity highly depends on goblet cell and mucin secretion (►**Fig. 1**).

Calcium Associated Mucin Secreting Pathways

The Ca^{2+}-activated transient receptor potential (TRP) channels TRP cation channel subfamily M member 4 (TRPM4) and TRPM5 have great biological significance: both are vigorously activated by intracellular Ca^{2+}, which also helps to study their functional properties with patch-clamp recording.[33–36] There are two types of mucin secretion system, one is baseline and the other stimulated.

Baseline Mucin Secretion

In the absence of an external stimulus, TRPM4/TRPM5 is not active. Under these conditions, the cells control mucin secretion by coupling the function of intracellular calcium oscillations to potassium voltage-gated channel interacting protein 3 (KChIP3). The ryanodine receptor-mediated release of calcium from the endoplasmic reticulum raises the intracellular calcium threshold, loading calcium onto KChIP3 and triggering its release from the mature granule, which then fuses to the plasma membrane. This procedure is independent of low affinity calcium sensor synaptotagmin 2 and used by cells to control mucin secretion in the absence of extracellular calcium.[33]

Stimulated Mucin Secretion

When cells are stimulated (for example, by ATP or IL-13), there is a rapid burst of Ca2+ released from the endoplasmic reticulum (ER), which activates TRPM4/TRPM5. Once activated, TRPM4/TRPM5 permeates the cytoplasm with Na. The local increase in Na concentration in the proximity of Na/Ca2 exchanger 2 triggers them to act in reverse mode and pump calcium into the cells. This increases local Ca2+ concentration and engages the low affinity calcium sensor synaptotagmin 2 to promote fusion of mucin granules to the plasma membrane.[30] This procedure accounts for a rapid burst in mucin secretion under conditions such as exposure of cells to exogenous stimuli. Na^+/Ca^{2+} exchanger (NCX2) works in conjunction with TRPM4, and perhaps TRPM5, Na+ channels to control Ca2+-mediated secretion of both MUC2 and MUC5AC from HT29-18N2 colonic cancer cells.[30]

Cantero-Recasens et al.[30] found that blocking the activity of TRPM4 or NCX proteins abrogated MUC5AC secretion from differentiated normal bronchial epithelial (NHBE) and tracheal cells from patients with cystic fibrosis (CFT1-LC3 cells). Adenosine-5'-triphosphate-dependent mucin secretion by controlling calcium entry through TRPM4/TRPM5 and NCXs may be true. Colon cells express both TRPM4 and TRPM5 sodium channels, and only NCX2, while airway cells express TRPM4 and all three NCXs. The study[30] also revealed that knockdown of TRPM4, TRPM5, and NCX has a greater effect on the physiologically-secreted MUC2 by colonic cells (HT29-18N2) and MUC5AC release from the airway cells (NHBE and CFT1-LC3) respectively.

Ca^{2+} Dynamics and What are Ca2+ Oscillations?

Calcium ion oscillations are often associated with the propagation of Ca^{2+} waves within the cytosol, and sometimes between adjacent cells.[37] Calcium ion oscillation is vital in case of mucin. Along with other medications, it can prove to be a good therapeutic support in case of PMP in order to inhibit mucin secretion and its overload. A study found that KChIP3-depleted cells secreted 2.5 times more mucin at baseline but had no effect on agonist ATP-induced (stimulated) MUC5AC secretion. Overexpression of KChIP3 (KChIP3-GFP-labeled cells) decreased baseline MUC5AC secretion by 30% while having no effect on ATP-dependent MUC5AC secretion. Under physiological conditions, colonic goblet cells also secrete MUC2.[38,39] Thus, the obvious question which arises is whether KChIP3 is involved in baseline MUC2 secretion.

It was found that MUC2 secretion was significantly influenced by KChIP3 levels. In comparison to control cells,

KChIP3-KD cells had a 5.7-fold increase in baseline secretion, while KChIP3-GFP cells had a substantial decrease (70.2 percent reduction compared to control cells). These findings suggest that gel-forming mucin (MUC2) secretion from colonic goblet cells might follow a similar mechanistic pathway as that of MUC5AC. KChIP3-knockdown cells had substantially less mucin granules than control cells, according to these findings: In comparison to control cells, KChIP3-GFP-overexpressing cells displayed a drastic accumulation of apical mucin granules, as measured by an increase in the size of MUC5AC-positive particles. Now, Ca^{2+} oscillations might also have some connection with KChIP3 in somewhat similar fashion as they had a relation with TRPM4/TRPM5 and NCX.

Is KChIP3's Function Regulated by Intracellular Ca^{2+}Oscillations?

KChIP3 might be the link between Ca^{2+} oscillations and mucin secretion. There can be two possibilities: either Ca^{2+} oscillations control KChIP3 activity to regulate MUC5AC and MUC2 secretion or KChIP3 affects MUC5AC and MUC2 baseline secretion by controlling Ca^{2+} oscillations. The results of a study suggested that intracellular Ca^{2+} oscillations are key to baseline mucin secretion and that in the absence of these Ca^{2+} signals, KChIP3 disengages its function as modulator of baseline mucin secretion. Secondly, to test whether the link between KChIP3 and Ca^{2+} oscillations to regulate baseline mucin secretion relates to the Ca^{2+} binding capability of KChIP3. The observations from an earlier study suggested that intracellular Ca^{2+} oscillations under control- unstimulated- conditions regulate baseline Mucin secretion and that KChIP3 acts as a brake for mucin secretion. Knocking down KChIP3 increases while overexpression of KChIP3 decreases baseline mucin secretion. In addition, the role of KChIP3 in mucin secretion depends on basal intracellular Ca^{2+} signals and the ability of KChIP3 to sense such Ca^{2+} signals. Under conditions of low expression of KChIP3 (therefore, a reduced brake capability and, consequently, higher baseline mucin secretion), there is no further effect on secretion, even with an increase in Ca^{2+}oscillations.[40–42]

On the contrary, overexpression of KChIP3 (increased brake capability) inhibits secretion, and this effect is reverted by increasing Ca^{2+}oscillations, mainly in cells overexpressing KChIP3-GFP, but not KChIP3-MUT. We, therefore, conclude that both Ca^{2+}oscillations and KChIP3 function in the same pathway of baseline mucin secretion and Ca^{2+}oscillations likely control KChIP3 function. These results suggest that Ca^{2+}oscillations generated in goblet cells are sensed by KChIP3 to control baseline mucin secretion. Calcium ion oscillations control baseline mucin secretion in colonic cells, KChIP3 links Ca^{2+} oscillations to mucin secretion. In neurons, KChIP3 alters ER calcium content and RYR-mediated Ca^{2+}-induced Ca^{2+}release (CICR) by direct interaction with RYR receptors.[42–44] Thus, loss of KChIP3 causes mucin hypersecretion in vivo, thus inducing KChIP3 and inhibiting TRPM4/TRPM5 and NCX. Induction of KChIP3 will increase the brake effect on calcium-induced muci secretion, and inhibiting of

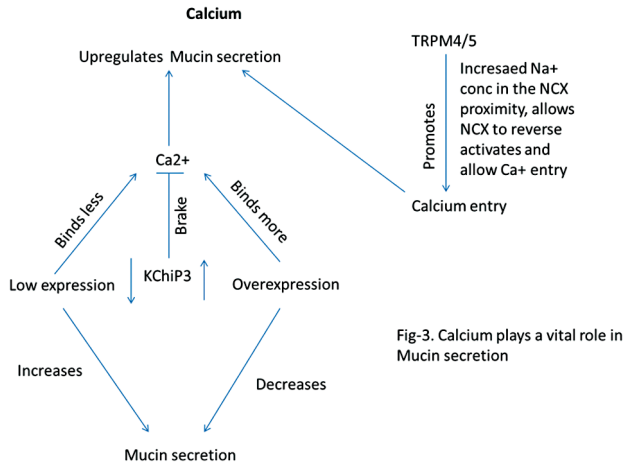

Fig-3. Calcium plays a vital role in Mucin secretion

Fig. 2 Calcium plays a vital role in mucin secretion.

TRPM4/TRPM5 and NCX will block Na+ dependent Ca^{2+}entry. A dual inhibitory effect will, thus, be created to stop mucin secretion (►**Fig. 2**).

Dexamethasone

Dexamethasone (Strong Inhibitor of MUCIN2 Synthesis)

Dexamethasone (Dex)is a synthetic glucocorticoid, and glucocorticoids reduce airway inflammation and mucin secretion.[44,45] The inflammatory milieu of the intestinal tract is highly conducive to MUC2-promoter upregulation.[45–47] Thus, inhibiting the inflammatory responses may directly target MUC2 and other mucins. The basal MUC2 mRNA expression in mucin-secreting human colon cancer cell line (LS174T cells) was inhibited by various concentrations of Dex, with persistent inhibitory effect. In the subcutaneous LS174T (NaB was used to stimulate MUC2 production in LS174T cells) murine xenograft model, a significant reduction in tumor volume at days 14, 21, and 28 was noticed in animals treated with Dex. In the intraperitoneal PMP xenograft model, Dex treatment showed a significant reduction in mucinous tumor mass. At days 13 and 36 after chronic treatment with Dex, serial measurements of xenograft weight gradually decreased. Immunofluorescence analysis of samples from PBS- and Dex-treated animals at day 36 demonstrated a nonsignificant but gross reduction in MUC2 protein.[44,45] Glucocorticoids or Dex (synthetic glucocorticoid) may directly inhibit MUC2 production via glucocorticoid response elements (GRE) in the MUC2-promoter region or indirectly via transrepression of inflammation-associated transcription factors, including NFκB or AP-1.[47,48] Dexamethsone may become a promising drug for the inhibition of mucin production.

Methyl 6-thio-6-deoxy-α-D-galactopyranoside (reducing agent - mucolytic nature)

Mature mucins are composed of two distinct regions: The amino- and carboxy-terminal regions, which are very lightly glycosylated, but rich in cysteines. The cysteine residues

participate in establishing disulfide linkages within and among mucin monomers. The thiols of the cysteine domain form disulfide bonds among themselves under oxidative stress forming disulfide crosslinks.[48–50] Data bring evidence that enrichment with a protein made of CYS domains stiffens the mucin network to provide a more impermeable and protective mucus barrier than mucus without such enrichment.[50,51]

It suggests that cysteine plays an important role in sol-to-gel transformations of mucin, which is bad in case of PMP. Breaking disulfide bonds can be a good mucolytic effort, which will transit mucin from gel or to fluid form or may soften the existing stiff mucins in the intraperitoneal region. Targeting mucin disulfide cross-links using current thiol-amino structures such as *N*-acetylcysteine (NAC) requires high drug concentrations to have mucolytic effects. Therefore, a thiol-carbohydrate structure (methyl 6-thio-6-deoxy-α-D-galactopyranoside) is synthesized, and it was found that it had stronger reducing activity than NAC and more potent and fast-acting mucolytic activity in cystic fibrosis sputum.[49,52] A combination of these two components will suppress future production of mucin along with lysis of existing mucin (►**Fig. 3**).

Naltrexone (Low-dose Naltrexone-LDN)

There are a number of diseases, such as fibromyalgia, CD, and multiple sclerosis, for which LDN is used as an antiinflammatory drug. It inhibits cellular proliferation of T and B cells and blocks toll-like receptor 4, resulting in an analgesic and antiinflammatory effect. Low-dose naltrexone causes transient blockade of opioid receptors, centrally resulting in a rebound of endorphin function which may attenuate pain in fibromyalgia.[51,53]

Nerve cells of the brain, the spinal cord and the digestive tract show the presence of opioid receptors (μ-, κ-, δ- and ζ-opioid receptors). Among these receptors, the ζ-receptor, also known as opioid growth factor receptor (OGFr), expresses inside or on the surface of the immune cells, which indicates that agonists and antagonists of OGFr can play immunoregulatory functions. Low-dose naltrexone has a strong blocking effect on OGFr; it intermittently (irregular dose) blocked OGFr and significantly inhibited the growth of neuroblastoma in tumor-bearing mice. Low-dose naltrexone could modulate the function of immune cells such as bone marrow dendritic cells (BMDCs) and macrophages.[52–56]

Naltrexone simultaneously blocked non-opioid receptors such as TLR-4 in macrophages and microglia.[55–58] Low-dose naltrexone increased the concentration of IL-2 and induced the secretion of tumor necrosis factor (TNF)-α in BMDCs, and it can also improve the expression of MHCII, CD40, CD83, CD80, and CD86 molecules on the surface of BMDCs. Thus, LDN has effective immunomodulatory functions that might become quite helpful in PMP. It relieves the neurotoxicity of glutamate on nerve cells by inhibiting inducible nitric oxide synthase (iNOS) activity[57,59] and reducing inflammation.

Low-dose naltrexone (can also downregulate the expression of pro-apoptotic proteins by activating apoptotic pathways.[58,60]

In ovarian tumor-bearing mice, LDN caused intermittent opioid receptor blockade and upregulated the expression of OGF and OGFr,[59,61] inhibiting tumor progression in a cytotoxic manner by reducing DNA synthesis and angiogenesis rather than altering cell survival. Intermittent LDN for a short period of time (4–6h) followed by immediate LDN clearance, there was a window period of 18 to 20h during which time the tumor cell growth was significantly inhibited.[54,62] During this window, the numbers of endogenous OGF and

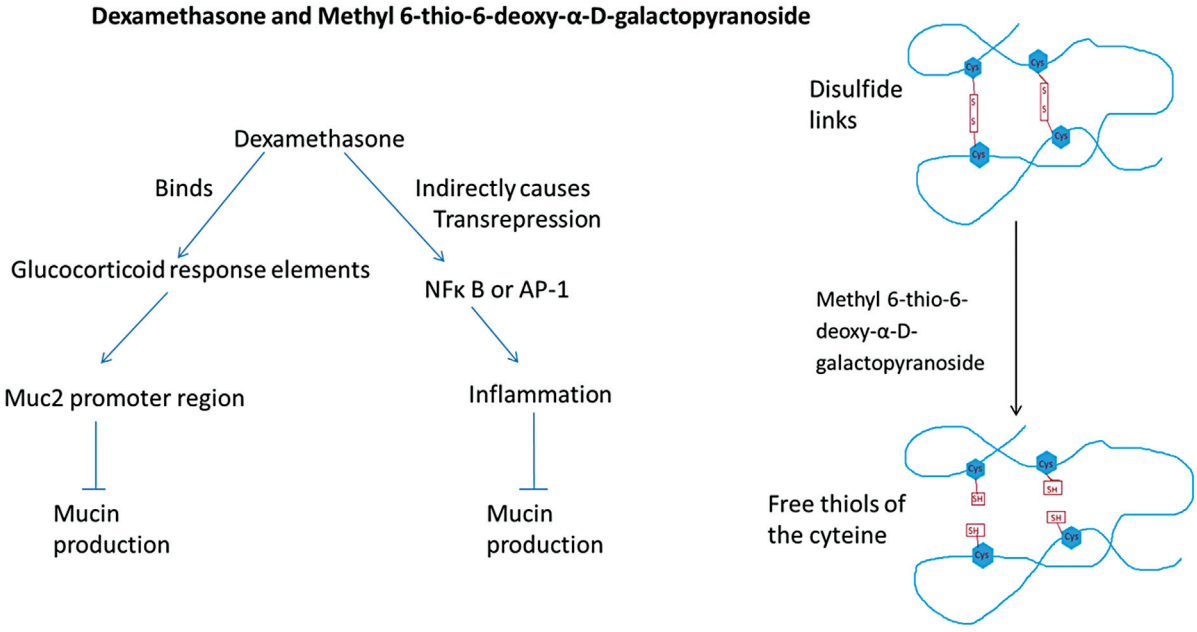

Fig. 3 Combination of dexamethasone and methyl 6-thio-6-deoxy-α-D-galactopyranoside can reduce mucin production and lysis of the produced mucin.

intracellular OGFr in tumor cells were detected to increase but still blocked.

Therefore, both low and intermittent doses are essential, LDN showed similar effect on other ovarian cancer cell lines and breast cancer cell lines, along with this it also also alleviates the adverse effects of chemotherapeutic agents such as paclitaxel by protecting non-tumor cells from death.[59,61]

LDN Combination Drugs

Low-dose naltrexone- (and α-lipoic acid (ALA/N)-treated [60,61,63,64] patients with metastatic or non-metastatic pancreatic cancer achieved long-term survival without any adverse effects. The use of hydroxycitric acid (HCA) + α-lipoic acid (α-LA) + LDN was safe and effective for the treatment of end-stage cancers and was capable of modulating the metabolism of various cancers. In addition, cells pretreated with LDN are more sensitive to the cytotoxic effects of common chemotherapeutic drugs. Low-dose naltrexone not only functions as a monotherapy for cancer but is also effective in combination with other agents, such as aged garlic extract,[62,65] vitamin D,[63,66] and panobinostat, to inhibit tumor growth. A research team has used the combination of low-dose naltrexone and methionine enkephalin MENK (also called opioid growth factor [OGF]) that inhibited DNA replication of pancreatic tumor cells and stimulated activation and proliferation of immune cells thus promoting the body to heal itself.[64,67]

A study inferred that LDN has protective effect against 2,4-Toluene diisocyanate-induced toxicity in albino rats by depleting goblet cells. Another study found that LDN exhibits protective effect against Crohns Disease (CD) with abnormal mucin secretion. There are no study which directly interprets that LDN inhibits mucin secretion in CD. Moderate expression of MUC2 and MUC3 (50.0% and 32.1%) and high expression of MUC4 and trefoil factor 3 (TFF3) in the colon mucosa were observed in all patients with CD.[66,68] Though both CD and PMP are different diseases, both have two things in common, one is mucin expression and the other is inflammation, and if LDN is causing remission in CD, it may also show positive remission in PMP.

A study in adult patients of CD reported that 30% (5/18) of LDN-treated patients achieved clinical remission at 12 weeks compared to 18% (3/16) of placebo patients, a difference that was not statistically significant (RR 1.48; 95% CI 0.42–5.24). Sixty-one per cent (11/18) of LDN patients achieved a 100-point clinical response compared to 31% (5/16) of placebo patients.[67,69] Naltrexone directly improves the epithelial barrier function by improving wound healing and reducing mucosal ER stress levels,[68,70] which is also present in case of PMP. If LDN has all the functions, such as antitumor, anti-inflammatory, immunomodulatory as well as being able to deplete goblet cell, then it can surely be tested for treatment against PMP (►**Fig. 4**).

DNMT and TET

The first reports on DNA methylation changes in cancer described global loss of methylation, which has been suggested to drive tumorigenesis through activation of oncogenic proteins or induction of chromosomal instability. In this context, reducing DNA methylation was viewed as a tumor-promoting event rather than a promising cancer therapy. The idea of inhibiting DNA methylation

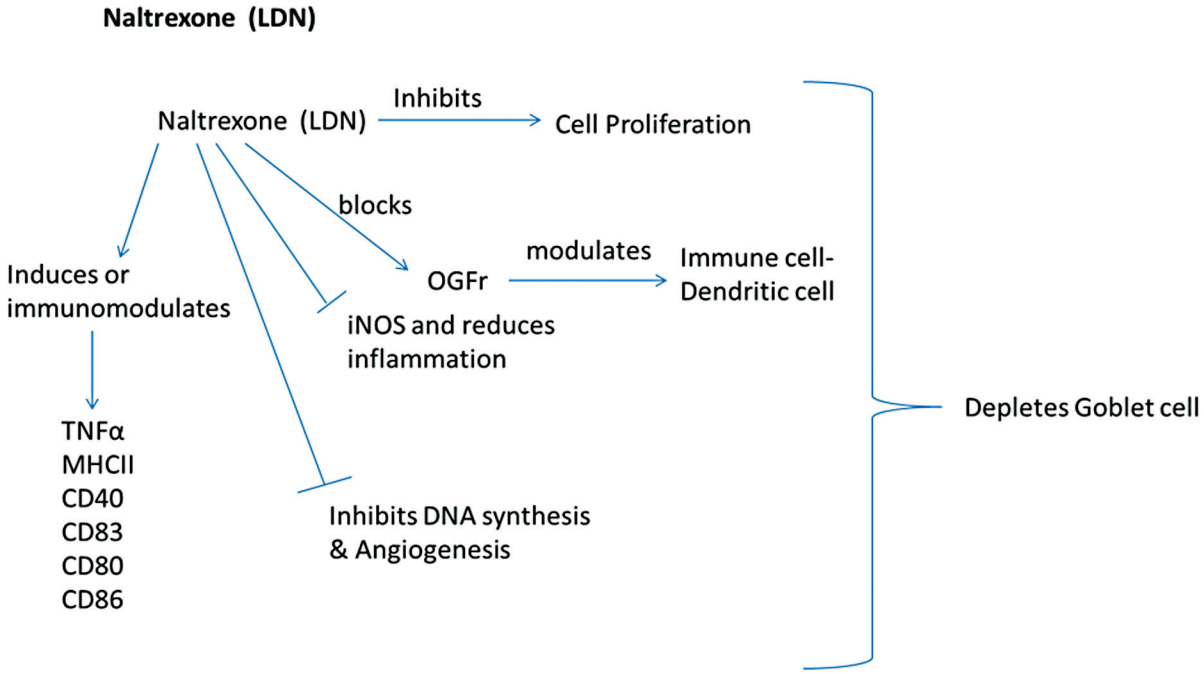

Fig. 4 Naltrexone depletes goblet cell and reduces cell proliferation via immune modulation, regulating angiogenesis, and controlling DNA synthesis.

therapeutically emerged from subsequent studies showing that, in parallel to global decreases in methylation, several genes (including many critical to the tumor phenotype) displayed gains of methylation in their promoters during tumorigenesis, a process associated with epigenetic silencing of expression and loss of protein function This led to revival of interest in drugs discovered decades ago to be potent inhibitors of DNA.[69,71]

The strong expression of MUC2 in the normal human goblet cells is associated with the average methylation of about 50% at every investigated CpG site of the MUC2 promoter of the human mucinous colorectal carcinomas. In contrast, MUC2 promoter in the non-expressing normal columnar cells and in the non-mucinous carcinoma tissue is methylated to nearly 100%. These data show that (i) low methylation of MUC2 promoter is associated with MUC2 expression in vivo. This clearly suggests that increased methylation in the promoter region will reduce mucin expression, and a balanced methylation pattern is essential for normal or increased mucin expression, which is majorly controlled by TET, a demethylating enzyme which seems to be responsible for actual balance. The TET methylcytosine dioxygenase family of enzymes induces active demethylation and creates a balanced methylation.[70,72]

Ten-eleven translocation enzymes oxidize 5-methylcytosine (5-mC) to 5-hydroxy-mC (5-hmC) (this is hydroxymethylation), which is modified through several suggested mechanisms including deamination and decarboxylation, ultimately leading to base-excision repair and replacement with an unmethylated cytosine. Ten-eleven translocation 1 is the most prominent member of the TET family, and previous studies showed that knockdown of TET1 results in increased global methylation in mice.[71,73] Other suggested mechanisms for active DNA demethylation are also present, but TET seems to be the most targetable.

Cells possess standard methylation profiles by maintaining a balance between DNA methylation and demethylation processes. Induction of DNMT may lead to hypermethylation and, thereby, reduced mucin expression; But TET still remains functional and may keep reversing the process of methylation. So, inducing DNMT can be helpful in reducing mucin expression, but inhibiting TET will give better results. Inhibiting TET along with induction of DNMT will be best.

There are studies which explain that TET1 is important in intestinal epithelium differentiation, and this is correlated with hydroxymethylation (which is a step just before demethylation). The same study also confers that colon cancers exhibit decreased hydroxymethylation and altered gene expression. When colon adenocarcinoma CaCo2 cells were treated with doxycycline (inducer of TET) for 96 hours, these cells had slower growth, and there were 300 genes with altered expression, and 60% of the genes with increased expression. It is quite possible that induction of TET and hydroxymethylation reduces cell growth to a certain extent, but it also increases the expression of 60 % of the genes, with mucin being one of them.[72,74] So in case of PMP, TET enzymes need to be inhibited in order to reduce cancerous growth and mucin secretion.

Abnormal methylation has been postulated to inactivate tumor suppressor genes through cytosine methylation and activate oncogenes through cytosine hydroxymethylation or demethylation. Thus, inhibition of TET may stop oncogenes activation as well as increased methylation may suppress expression of certain genes, which can even be mucin.[73,75] A study described TET inhibitors as possible novel anti-cancer drugs. They explain how inhibitors of metabolic enzymes, such as fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and succinate dehydrogenase (SDH), may become new avenues for anti-cancer drug research. These enzymes are in the citric acid cycle, and are frequently mutated in cancer, leading to the production of alpha ketoglutarate (α-KG), a cofactor for TET activity. The study by Chua et al., has identified a new class of cytosine-based TET enzyme inhibitor named as Bobcat339. This potent inibitor had a mid-μM IC50s for TET1 and TET2 without inhibiting DNMT3a. It reduced 5hmC abundance in the DNA of cultured neurons.[74,76] According to the current review, the future challenges include finding compounds that can induce DNMT or inhibit TET only in goblet cells. Targeted delivery of the drug is essential so that it will not hamper the methylation balance of other cells (►**Fig. 5**).

Retinoic Acid (RA) and Retinoic Acid Receptors (RARs) Antagonist in PMP

Retinoids are a class of compounds structurally related to vitamin A, including natural and synthetic compounds. A series of retinoids has been found to be clinically useful in treating dermatological and oncological diseases and are responsible for the structure and function of a wide range of inflammatory, immune, and structural cells. It also regulates epithelial cell differentiation, proliferation, and morphogenesis of the lung. Retinoids exert their biological effects through a series of nuclear receptors, ligand-inducible transcription factors belonging to the steroid / thyroid receptor superfamily. Retinoid receptors are divided into two families: RARs and retinoid X receptors (RXR), each of which consists of three distinct subtypes (α, β, and γ).

Compounds having RAR antagonist activity inhibit cell proliferation and angiogenesis but induce cell differentiation.[75,77] Studies describe several classes of RAR antagonists, including the RARα selective antagonists.[76,78] Mucins are a family of glycoproteins secreted by epithelial cells, including epithelial cells of the respiratory, gastrointestinal, and female genital tracts. The viscoelastic properties of mucus are attributed to mucin, and, altogether, eight mucin genes exist.[77,79] Many airway diseases, such as chronic bronchitis, chronic obstructive pulmonary disease, bronchiectasis, asthma, cystic fibrosis, and bacterial infections, are characterized by mucin overproduction.[78–82]

The reported effects of retinoid on mucin expression are conflicting. An investigator reported that vitamin A (retinol) downregulated the expression of the MUC2 gene in tracheobronchial epithelial cells,[81,83] while others have shown that a retinoid-supplemented culture of normal human tracheobronchial epithelial cells has an increased MUC2 and

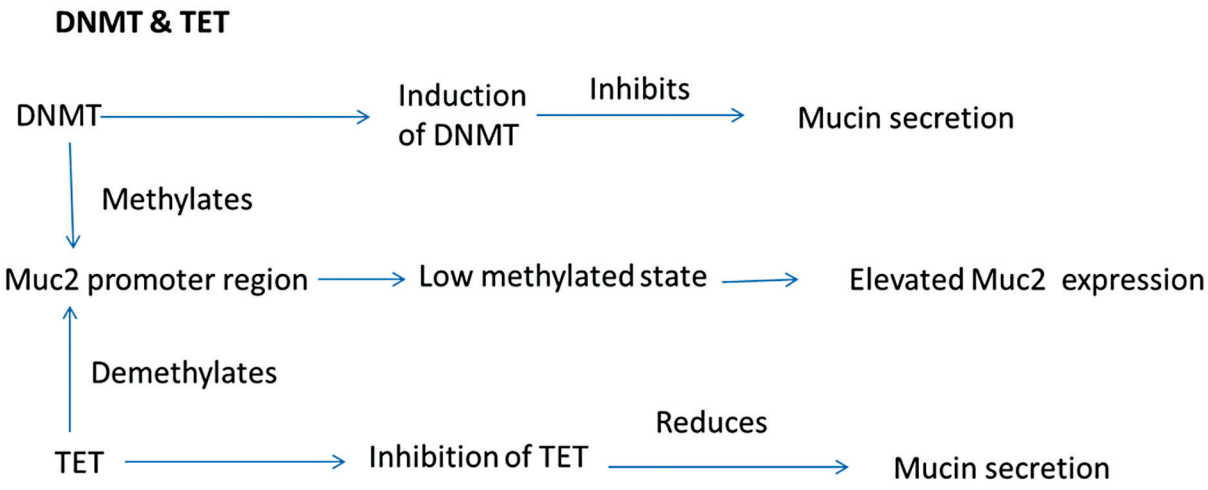

Fig. 5 Induction of DNA methyltransferase and inhibition of ten-eleven translocation anzyme can possibly reduce mucin synthesis and secretion.

MUC5AC mRNA expression.[82,84] Retinoic acid is required for mucociliary differentiation of normal human tracheobronchial epithelial cells. Absence of retinoic acid causes epithelium to become exfoliative, and secretion of mucin is reduced.[83,85]

Similarly, some studies show that reduced RXRα signaling increases conjunctival monocyte infiltration, IFN-γ expression, and goblet cell loss; this clearly explains that retinoic acid is responsible for the induction of certain inflammatory factors, which, again, induce an increase in the number of goblet cells. Contrastingly, some evidence suggests that dry-eye therapies that suppress IFN-γ expression preserve conjunctival goblet cell number and function[84,86] Therefore, studies evidence a strong relation between IFN- γ expression and goblet cell induction. It seems like IFN-γ and goblet cells are inversely proportional, because increased IFN-γ expression reduces goblet cells, and suppression of IFN-γ preserves the number of goblet cells.

Interestingly, The study by Xiao et al., 2018, tested the hypothesis that mouse conjunctival goblet cells produce biologically active retinoic acid (RA) that suppresses CD86 expression and IL-12 production by myeloid cells (there is not much or any difference between conjunctival esophageal or intestinal or peritoneal goblet cells). This suggests that RA originated from goblet cell may function in maintaining loss of conjunctival goblet cells and may contribute to increased Th1 priming in dry eye.[85,87] Thus, RA cannot be responsible for goblet cell loss. The second interesting fact is that RA acts on intestinal leukocytes to modulate their lineage commitment and function. The lack of RARα signaling in intestinal epithelial cells (IECs) results in deregulated specification of epithelial lineage, allowing increased goblet and Paneth cells.[86,88]

Studies indicate that inhibitors of mucin synthesis and goblet cell hyperplasia includes the inhibitors of epidermal growth factor (EGF), receptor tyrosine kinase inhibitors, p38 mitogen-activated protein (MAP), kinase inhibitors, MAP kinase kinase/extracellular signal-regulated kinase (MEK/ERK) inhibitors, human calcium-activated chloride (hCACL2), channel blockers, and RXRα antagonists. This clearly explains that RAR antagonists can reduce goblet cell hyperplasia[87,89] Now, it is evidently clear that RA supports goblet cell hyperplasia and mucin secretion, whereas RAR antagonists can effectively reduce goblet cell hyperplasia and mucin secretion. However, the mechanism that mediates the effects of retinoic acid on goblet cell production or differentiation of epithelial cells into goblet cells is still unknown.[88,90]

The study by Obinata et al., 2011 and Fuji et al., 2017 have found that in chick embryonic cultured skin, RA was found capable of inducing epidermis to transdifferentiate into mucosal epithelium with goblet cells. This study shows that TG/2 and Gbx1 along with TGF-beta2 pathways have differential influence strong enough to utilize RA and convert epithelial cells into goblet cells.[89,91] Since RA is exclusively involved in the maintenance of the goblet cell population, it can even convert epithelium into goblet cells. Thus, it is considered the best target in diseases associatedwithmucin hypersecretion and antagonising RAR may surely be effective to reduce RA function in PMP-like diseases, which have a huge burden of mucin.

A mice model with allergic rhinitis (AR) shows that RORC inhibitor group significantly reduced the symptom score (4.02 ± 0.97 vs 8.50 ± 1.76, $t = 7.050$, $p < 0.01$) with mast goblet cells significantly reduced, and reduced infiltration of inflammatory cells in the inherent mucosa. Interleukin 17 and sIgE in serum decreased, IFN-γ increased, and HIF-1α and VEGF protein in the nasal mucosal tissues of AR mice were significantly reduced.[90,92] Therefore, RA is essential for goblet cell maintenance and mucin production, and it definitely does so via inflammatory pathways involving molecules such as IFN-γ, IL-17 and hypoxia -inducing factor 1 alpha (HIF-1 α).

Oral administration of NEt-4IB (retinoid X receptor partial agonist) significantly suppressed AHR and inflammatory cell accumulation in the airways and attenuated the levels of

TNF-α in the lung and IL-5, IL-13, and NO levels in bronchoalveolar lavage (BAL) fluid and the number of periodic acid Schiff (PAS)-positive goblet cells in lung tissue. Treatment with NEt-4IB also significantly suppressed NF-κB expression.[91,93]

The RAR antagonists, particularly RARα selective antagonists disclosed herein, inhibit mucin overexpression associated with inflammation and mucus secretion associated with systemic epithelial cell inflammation and similar conditions. The use of the RAR antagonists in combination with another active ingredient to improve mucin clearance may include sodium channel blockers (e.g., amiloride) or antibiotics (e.g., duramycin, nisin, or subtilin).

Mechanism of Goblet Cell Production from Stem Cell Involves RA

In the small intestine, crypt base columnar (CBC) stem cells produce progenitor cells that are variably induced by atonal homologue-1 (atoh1) or hairy enhances of split-1 (hes1) to differentiate into the various secretory cell types or into absorptive enterocytes, respectively.[92–95] Both Atoh-1 and Hes-1 repress and regulate each other. Atoh1 allows intestinal expression of the neurogenin3 (neurog3) transcription factor, which induces differentiation of enteroendocrine cells. Growth factor independent factor 1 (Gf1) inhibits this pathway to permit differentiation into goblet/Paneth cells.[94]

The study by Taseff et al., 2017 has presented an effective model of all-trans retinoic acid (ATRA)-induced differentiation of HL-60 cells. Knockout analysis suggested that the growth factor independence-1 (Gfi1) and PPARg were critical to the ATRA-induced differentiation program, as we have earlier described that Gfi1 inhibits Atoh-1 and Hes-1 pathway to permit the differentiation of CBC stem cells only into goblet/Paneth cells. Thus, ultimately, inhibiting RA or retinoid function by inhibiting RAR using antagonist will definitely work wonders in case of goblet cell depletion and mucin secretion (►**Fig. 6**).[95]

Conclusion

Boldine, a primary alkaloid that can inhibit MPO was directly proportional to goblet cell and mucin secretion. Glial fibrillary acidic protein was also associated with inflamed colon and Crohn disease. These diseases are similar to PMP in relation to mucin secretion. Boldine was also noticed to inhibit GFAP, oxidative stress, and to modulate immune function. It also acts as antiproliferative agent, with cell-cycle arrest at G2/M phase and apoptotic property. Therefore, it can be useful in PMP. Amiloride may also be a good candidate drug to treat PMP. It has the important function of blocking NHE, which seems to be an important part of the goblet cell and its function. Thus, using amiloride to block NHE will both block the function of existing goblet cells and inhibit their differentiation.

NCX2 works in conjunction with TRPM4, and perhaps TRPM5, and Na+ channels to control Ca^{2+}-mediated secretion of both MUC2 and MUC5AC from HT29-18N2 colonic cancer cells. Adenoside triphosphate-dependent mucin secretion by controlling calcium entry through TRP M4/TRPM5

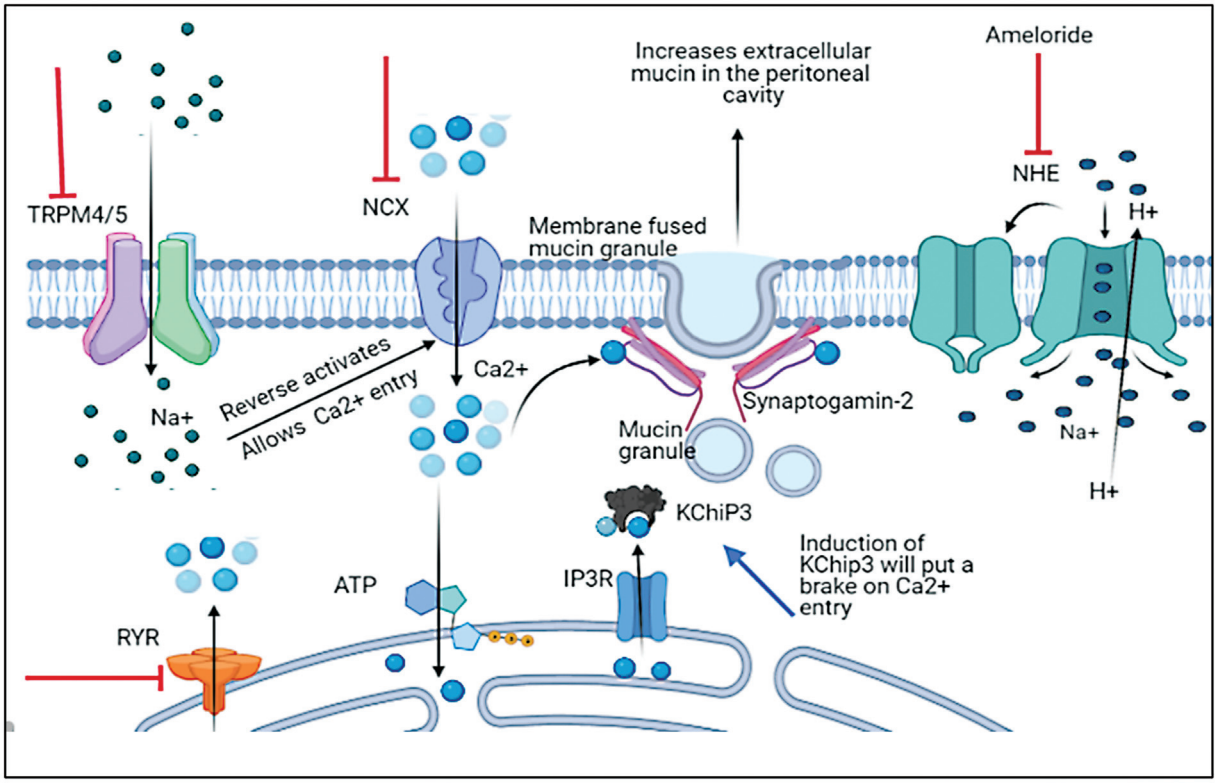

Fig. 6 The red inhibitory lines and blue induction line show targets to be inhibited and induced in order to reduce mucin production and its secretion.

and NCXs is possibly true. Both TRPM4 and TRPM5 Na+ channels, and only NCX2, are expressed in colon cells. The study by Cantero et al., 2019 found that internal Ca^{2+}stores (especially the ER) are the source of Ca^{2+}oscillations in goblet cells. Importantly, RYRs are involved in the generation and maintenance of these oscillations.[30]

We have discovered that ryanodine receptor-dependent intracellular Ca^{2+} oscillations affect the dissociation of the Ca^{2+}-binding protein, KChIP3, encoded by the *KCNIP3* gene, from mature mucin-filled secretory granules, allowing for their exocytosis. Increased Ca^{2+} oscillations, or depleting KChIP3, lead to mucin hypersecretion in a human differentiated colonic cell line, an effect reproduced in the colon of *Kcnip3*$^{-/-}$ mice. Conversely, overexpressing KChIP3 or abrogating its Ca^{2+}-sensing ability increases KChIP3 association with granules and inhibits baseline secretion. Therefore, KChIP3 emerges as the high-affinity Ca^{2+} sensor that negatively regulates baseline mucin secretion. We suggest KChIP3 marks mature, primed mucin granules, and functions as a Ca^{2+} oscillation-dependent brake to control baseline secretion.

Low-dose naltrexone can be a good immunomodulatory drug which can inhibit inflammation as well as antitumor with very little or no research on its effect on mucin expression, but some studies have shown depletory effect of LDN on goblet cell. Low methylation state of MUC2 promoter region was associated with increased mucin expression. DNA methyltransferase is responsible for methylation and TET is responsible for demethylation. So, Induction of DNMT and inhibition of TET enzyme may inhibit mucin expression with reduced growth of PMP. In one aspect, the present scenario and literature mining provides that the use of a RAR antagonist for inhibiting mucin production and goblet cell synthesis in a mammal will highly be effective instead of using RA. The RAR, RXR, or ROR antagonist may work effectively in depleting goblet-cell synthesis both by inhibiting the stem cell pathway and by epithelial transdifferentiation pathway leading to inhibition of mucin secretion.

Conflict of Interest

The authors have no conflict of interests to declare.

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