CFTR and Gastrointestinal Cancers

Subjects: Oncology

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Cystic Fibrosis (CF) is a disease caused by mutations in the *CFTR* gene that severely affects the lungs as well as extra-pulmonary tissues, including the gastrointestinal (GI) tract. CFTR dysfunction resulting from either mutations or the downregulation of its expression has been shown to promote carcinogenesis. An example is the enhanced risk for several types of cancer in patients with CF, especially cancers of the GI tract. CFTR also acts as a tumor suppressor in diverse sporadic epithelial cancers in many tissues, primarily due to the silencing of CFTR expression via multiple mechanisms, but especially due to epigenetic regulation.

Keywords: cystic fibrosis ; CFTR ; gastrointestinal cancers ; tumor suppressor gene

1. Introduction

Cystic Fibrosis is a monogenic disease caused by mutations in the *CFTR* gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. The CFTR protein is a cAMP-dependent anion transporter that transports chloride (Cl^-) and bicarbonate (HCO_3^-) ions across the apical plasma membrane of epithelial cells. Moreover, this protein regulates the activity of other ion channels, including epithelial sodium ion channels. Therefore, mutations or the absence of CFTR results in an imbalance of ions and fluids in the cells of the airways, intestine, pancreas, and other organs [2]. Mutations in CFTR are generally classified based on the nature of the dysfunction, including aberrant protein production, cellular processing or activity. Mutations in the *CFTR* gene fall into six basic classes. The most common pathogenic mutant form of CFTR is designated as F508del (a phenylalanine residue at site 508 is deleted) and this mutation is most common among Caucasians of northern European descent [3].

With the recent development of improved CF therapies, including CFTR modulator drugs, the life expectancy of patients with CF has increased substantially. However, one major challenge that still remains is the increased predisposition of patients with CF to cancer, as shown by many studies. CFTR has been found to act almost exclusively as a tumor suppressor in different cancer types and dysfunctional CFTR has been associated with the promotion of carcinogenesis [4]. Among the different cancer types, there is an increased risk of patients with CF for gastrointestinal (GI) cancers, in particular colorectal cancer (CRC), as the risk of CRC among patients with CF is increased by 6-fold [5][6]. Moreover, there is an even stronger risk of CRC in patients with CF who have undergone a lung transplant [7]. Colonoscopy is considered to be the most effective method for the screening of CRC in patients with CF.

2. Role of CFTR in Normal Physiological Conditions and Its Dysfunction in CF

The CFTR protein belongs to the ABC (ATP-binding cassette) class of transporters which are generally involved in transmembrane transport. The *CFTR* gene is transcribed into an mRNA transcript of 6128 nucleotides, which is then translated into a protein of 1480 amino acids. The CFTR protein is composed of two symmetrical halves with each half consisting of six membrane spanning domains joined by regulatory regions. The membrane spanning domains constitute an aqueous pore-like structure via which the Cl^- and HCO_3^- ions are transported across the plasma membrane down their electrochemical gradient. Ionic specificity is determined by the amino acids which line the pore-like structure. Pore opening and closure is usually controlled by the binding of ATP to the nucleotide binding domains present in the regulatory region. The flow of ions through this channel results in the development of an osmotic pressure, which drives the movement of water in the same direction [8]. In the intestine, CFTR protein is expressed at the apical surface of epithelial cells throughout the entire length of the intestine. In the small intestine, the expression of CFTR gradually decreases from the duodenum to the ileum. In the colorectum, a high expression of CFTR is observed in the proximal colon and cecum. In both the small and large intestine, the highest expression of CFTR is observed in the crypts where the intestinal stem cells are present [9][10].

Biallelic inactivating mutations in the *CFTR* gene result in the development of CF. The most severe clinical manifestations associated with CF are pulmonary inflammation and obstruction, ultimately resulting in pulmonary failure. However, the dysfunction of CFTR in extra-pulmonary tissues, such as the pancreas and biliary ducts, accounts for CF-linked diabetes and liver diseases [11]. Loss of CFTR in the intestine results in obstruction in proximal colon and ileum in infants and distal intestine in older patients. Moreover, patients with CF are also prone to develop celiac disease. CFTR dysfunction in the GI tract results in low intestinal pH, thick mucus deposition, and an impaired innate immune response. These mechanisms generally drive the local inflammation of the GI tract that is proposed to increase the likelihood of an early onset of GI cancers.

3. Involvement of CFTR in GI Cancers

3.1. Esophageal Cancer

CFTR dysfunction is associated with the development and progression of esophageal cancers, both esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), and all studies support a tumor suppressing role for CFTR. Association of CFTR with esophageal cancer has been summarized in **Table 1**.

Table 1. The role of CFTR in Esophageal Cancer.

Cancer Type	CFTR-Related Phenotypes
Esophageal Cancer	• Expression of CFTR was downregulated in ESCC tissues and was part of an autophagy-related gene prognostic risk signature in esophageal cancer patients [12]. • KEGG and STRING bioinformatics analyses identified CFTR as one of the top ten gene hub nodes that are dysregulated in esophageal cancer [13]. • CFTR expression was lower in tumor tissues as well as esophageal cancer cell lines. CFTR suppressed the expression of NF-κB-p65 and tumor growth in esophageal cancer cells. KD of CFTR in ESCC cells increased NF-κB pathway signaling and promoted invasive growth in vitro and cancer cell growth in vivo in a mouse xenograft model [14]. • CFTR overexpression in ESCC cells suppressed cell proliferation, migration and invasiveness while promoting apoptosis. IHC analysis of the invasive front of primary ESCC tumors showed an inverse relationship between CFTR expression and post-operative survival. Microarray gene expression profiling found that the p38 MAPK pathway was regulated by CFTR which governed ESCC progression [15]. • Epidemiological studies indicate that patients with CF are at a higher risk of esophageal cancer and gastroesophageal adenocarcinoma [5][6]. • Adults with CF have a 3-fold high risk of developing Barrett's esophagus which is a precursor for esophageal cancer [16]. • GWAS studies reported that the CFTR locus was a risk factor for both Barrett's esophagus and EAC [17].

3.2. Pancreatic Cancer

Pancreatic insufficiency due to pancreatic damage, loss of acinar cells, fatty replacement and interstitial fibrosis is a common problem in CF [18]. It is well known that chronic inflammatory pancreatitis (CP) is linked to an increased risk for pancreatic cancer. While the incidence of CP in patients with CF is relatively low (<2%), there are several studies that have found an association between CFTR mutations and both CP and pancreatic cancer. Hamoir et al. reported that mutations in the *CFTR* gene were linked to genetically determined CP and pancreatic cancer. They also found that pancreatic cancer was diagnosed at an earlier age in subjects with CFTR mutations [19]. This finding was consistent with that of McWilliams et al., who also reported a modest enhanced risk of pancreatic cancer diagnosed at an earlier age in patients whose tumors carried CFTR mutations [20]. CFTR has been reported to regulate the expression of MUC4, a glycoprotein implicated in tumor progression. Silencing of CFTR was found to enhance the expression of MUC4 in pancreatic cancer cells. Moreover, their expression profile demonstrated a negative correlation in these cells [21].

3.3. Hepatic Cancer

In patients without CF, the downregulation of CFTR expression was reported in a study by Chen et al., who studied abnormally methylated differentially expressed genes in HCC, using gene expression profiles available from the Gene Expression Omnibus (GEO) that identified CFTR as a hypermethylated, low expressing hub gene in HCC [3]. Similar downregulation of CFTR by promoter hypermethylation was reported by Moribe et al. in a study of 25 HCC samples compared with normal control tissues [22]. CFTR also plays an important role in the biliary tract, specifically in biliary epithelial cells (cholangiocytes), where it is expressed on the apical membrane. Maisonneuve et al. reported a higher risk of biliary tract cancers in patients with CF [5][6].

3.4. Gastric Cancer

To date there has not been a strong association between CFTR dysfunction and a risk for gastric cancer, either in patients with CF or in patients without CF.

3.5. Intestinal Cancer

CFTR mutations or the downregulation of CFTR expression has been strongly implicated in the development and progression of intestinal cancers of both the small and large bowel, particularly colorectal cancer, in patients with CF and patients without CF and in animal models of CFTR-deficiency. Indeed, CFTR's action as a tumor suppressor in the GI tract is considered strongest in the intestine. Maissoneuve et al. reported a 6-fold increased risk of CRC in CF patients based on a 20-year epidemiological study [5][6]. Since then, follow up studies have further supported a strong role for CFTR in prevention of CRC. **Table 2** highlights the involvement of CFTR in Intestinal cancer.

Table 2. Role of CFTR in Intestinal Cancer.

Cancer Type	CFTR-Related Phenotypes
Intestinal Cancer	• A 20-year epidemiological study found that patients with CF are at a 6-fold higher risk for CRC [5][6], a finding confirmed in a separate study of 99,000 adult CF patients [7]. Patients with CF are also at a higher risk of cancers in the small intestine, in particular, the duodenum. • Endoscopic screening studies reported a high incidence of early aggressive adenomas in patients with CF patients by age 40, with some having advanced to adenocarcinomas [23][24]. • Germline heterozygous carriers of CFTR mutations are at a higher risk of developing CRC [25][26]. • Germline heterozygous carriers of specific CFTR polymorphic variants are also at a higher risk for CRC, including in young adults [27] and in patients with familial serrated polyposis syndrome [28]. • CFTR also acts as a tumor suppressor and is downregulated in non-CF, non-germline, sporadic human CRC, with CFTR expression linked to DFS and better prognosis [28][29][30]. Expression of CFTR inhibited the proliferation, invasion and migration of CRC cells [31]. • CFTR's role as a tumor suppressor has been confirmed in CF mouse models, either in combination with *Apc*^{Min} mutations or in *Apc* wildtype mice [29]. • CFTR was reported to interact with AF6/afadin in regulating colon cancer metastasis [31]. • Loss of CFTR in the intestine is associated with increased Wnt/β-catenin signaling [32].

4. Potential Molecular Mechanisms Involved in the Regulation of CFTR in Cancer

CFTR has been found to act as a tumor suppressor in various cancer types and its expression is generally downregulated in tumors. There are different mechanisms which have been implicated in the regulation of CFTR expression. Much of the evidence for these mechanisms has been generated in studies in the intestinal tract, so it is very possible that novel mechanisms of CFTR regulation may yet be discovered in non-intestinal tract GI cancers.

4.1. Epigenetic Regulation

4.1.1. Promoter Hypermethylation

The expression levels of CFTR mRNA and protein were found to be silenced by epigenetic mechanisms, mainly hypermethylation of the CFTR promoter, in several of the major cancers where CFTR has been shown to act as a tumor suppressor.

4.1.2. Activity Involving CIS-Regulatory Elements (CREs)

Three-dimensional chromatin structure analysis revealed another epigenetic mechanism, *cis* regulatory elements (CREs) encoding for enhancers at the CFTR locus, that have been reported to regulate *CFTR* gene expression in intestinal and airway epithelial cells. CFTR lies within a topologically associated domain (TAD) flanked by CTCF and cohesion occupancy [33]. Within this TAD, specific CREs play a key role in the recruitment of activating factors to the CFTR promoter.

4.1.3. Silencing by MiRs

Another epigenetic mechanism that has been shown to modulate CFTR expression are miRNAs (miRs). An example is miR-125, which has been reported to be upregulated in primary CRC tumors in addition to metastases. In vitro, the overexpression of miR-125 in CRC cells enhanced invasion and migration. MiR-125 was shown to target CFTR, as supported by the results of a dual-luciferase assay. The targeting of CFTR by miR-125 enhanced the expression and the secretion of the urokinase plasminogen activator (uPA), along with the promotion of epithelial to mesenchymal transition (EMT) [34].

4.1.4. Action of Transcription Factors

Krüppel-like factors (KLFs) are a family of transcription factors (TFs) involved in development, proliferation and stem cell differentiation. Among them, KLF4 was found to be upregulated in CF respiratory epithelial cells compared to non-CF cells. Moreover, KLF4 overexpression negatively regulated the expression of CFTR in cells expressing wildtype CFTR. However, in the case of cells expressing F508del mutants, KLF4 had no impact on CFTR levels [35]. Another member of this TF family, KLF5, repressed the expression of CFTR in human primary airway epithelial cells and cell lines [36]. The depletion of KLF5 altered the higher order chromatin organization of the CFTR locus, thereby affecting its expression. Critical looping interactions, which are required for normal CFTR expression, were altered along with the redistribution of the H3K27ac active chromatin mark [37]. The KLF family of transcription factors, particularly KLF4 and KLF5, have been reported to promote GI cancer progression [38]. Therefore, KLF4/KLF5 may also be involved in the downregulation of CFTR expression in GI cancers.

4.2. Altered Signaling Pathways

CFTR expression and functioning has been shown to be regulated upstream by various signaling mechanisms, including the AKT and GSK3β pathways. These two pathways act in an opposing manner to regulate CFTR expression. AKT acts as a positive regulator while GSK3β negatively regulates CFTR expression [35]. CFTR has also been shown to be involved in the regulation of various downstream signaling pathways, including NF-κB and ERK in tumor cells [14][39].

5. Potential Mechanisms by Which CFTR Deficiency Promotes Carcinogenesis in the GI Tract

5.1. Influence on the Stem Cell Compartment

CFTR is generally expressed in the mucosal epithelia of the esophagus, stomach and both the small and large intestine. In liver, CFTR is primarily expressed in the intrahepatic biliary epithelium, and in the pancreas, CFTR expression is strongest in the small, intercalated ducts. Here, it is important to acknowledge that while CFTR expression has traditionally been described in the epithelia of tissues throughout the body, recent reports have demonstrated a much broader expression pattern for CFTR, for example, in the brain. Additionally, in the GI tract, CFTR has also been found to be expressed by enteric ganglia and in a variety of non-epithelial cells, such as fibroblasts, endothelial cells, neutrophils, lymphocytes, macrophages and mast cells, where its functions in these various cell types are yet to be characterized. Nonetheless, overall, in the GI tract, CFTR expression is strongest in the mucosal epithelia of the small and large intestine, principally in the crypts and localized to the base of the crypts that contain the intestinal stem cell compartment. Outside of the intestinal tract, much less is known about the identity and function of resident tissue stem cells. In the

intestine, CFTR expression in the crypt has been reported in the stem cell compartment, or immediately adjacent to it [32]. Thus, CFTR is well placed to influence intestinal crypt renewal and the activity of putative cancer progenitor cells.

5.2. Regulation of Wnt/β-Catenin Signaling

Wnt/β-catenin signaling is an essential mediator of intestinal tissue homeostasis, including stem cell survival, proliferation and differentiation, functions that are critical for normal intestinal cell renewal. Dysregulation of the Wnt/β-catenin signaling pathway is involved in ~90% of human CRC, both in early tumor initiation and in progression to invasive cancer [40]. CFTR deficiency in mouse small intestine was found to be linked to increased Wnt/β-catenin signaling [29] and tumors isolated from the small intestine of one year-old conditional intestinal-specific CFTR KO mice (*Apc* wildtype) had enhanced nuclear localization of β-catenin, as determined by IHC, along with elevated expression of Wnt/β-catenin target genes, such as *Cyclin D1* (*Ccnd1*), *Lgr5* and *cluster of differentiation 44* (*CD44*), as determined by RNA-Seq and q-RT-PCR [29]. This finding was confirmed in a separate study using CFTR constitutive KO mice, where KO mice showed enhanced intestinal stem cell proliferation and a significant increase in Wnt/β-catenin signaling [32]. Outside of the intestinal tract, Wnt/β-catenin signaling is also dysregulated in all of the other major GI tract cancers, including esophageal cancer [41], gastric cancer [42], liver cancer [43], and pancreatic cancer [44].

5.3. Disruption of Physical Barriers and Microbial Dysbiosis

CFTR is involved in multiple physical processes in the intestine that are critical for the maintenance of tissue homeostasis, including physical barriers to protect the single cell epithelial layer, the gut microflora and management of the innate and adaptive immune responses. Disruption of these processes is associated with tissue damage, inflammation and the creation of a favorable landscape for cancer development [45].

Two major physical barriers protect the colonic epithelium from contact with the microflora. First, the apical surface of the epithelium is covered by a dense inner and looser outer mucus layer. The loose outer layer contains abundant commensal bacteria, along with the potential presence of pathogenic bacteria [46]. The largely sterile inner layer is dependent on mucin2 (MUC2) proteins that are secreted by goblet cells and that, in turn, are dependent on bicarbonate ions (HCO_3^-) and water [47]. CFTR is directly involved in HCO_3^- efflux into the lumen [48] and also indirectly involved in H_2O efflux [49][50]. CFTR deficiency causes the mucus layer to become dehydrated and dysfunctional.

5.4. Proinflammatory Immune Cell Infiltration and Proinflammatory Signaling

Disruption of physical barriers not only permits access of bacteria to the epithelial layer of cells but also leads to infiltration of immune cells of the lamina propria into the epithelial layer, resulting in tissue damage and inflammatory signaling, mediated by the release of inflammatory cytokines by innate immune cells and the recruitment of proinflammatory cells of the adaptive immune response. Immune cell infiltration has been observed in patients with CF and in CF KO mouse models but clear histological evidence of tissue damage has not been observed [51].

CFTR deficiency is associated with the activation of proinflammatory signaling pathways that can promote cancer development. Activation of NF-κB signaling pathways has been observed downstream of CFTR deficiency in several tissues and cancers including in esophageal and intestinal cancers [30][52][53][54][55][56]. The expression of proinflammatory cytokines has been reported in several CFTR-deficient human CRC cell lines, including $CaCO^{-2}$ and HT-29 cells [54][57][58][59], and outside the GI tract CFTR has been shown to regulate the proliferation, migration and invasion of cervical cancer cells via inhibition of NF-κB signaling [60].

5.5. Altered Stress Responses

Due to high rates of metabolism in cancer cells, including CRC, oxidative stress and the production of reactive oxygen species (ROS) is increased. ROS can promote cancer development via damaging DNA, causing mutations, but ROS is also stressful for both normal and cancer cells, which can promote apoptosis.

6. Targeting Specific CFTR Mutations with Modulator Drugs, Implication for GI Tract Pathologies, including Cancer

CFTR modulator drugs function by either restoring or elevating the expression, stability and function of CFTR protein. These drugs now constitute an effective therapy for most patients with CF [61]. Based on their mode of action, the modulators have been divided into five main categories: potentiators, correctors, stabilizers, read-through agents, and amplifiers. R334W, R347P, and G551D are among the most common mutations that result in impaired CFTR channel

gating or conductance. Potentiators are compounds that increase CFTR channel open probability, thereby enhancing anion conductance.

The most common CFTR mutation is F508del, which is found in ~70% of patients with CF. For patients who are homozygous for this mutation, the combination of a single corrector (tezacaftor or lumacaftor) with a potentiator (ivacaftor) has been shown to improve clinical outcomes substantially. However, patients with CF who are heterozygous and have one F508del allele along with another mutation that accounts for non-responsiveness to these dual modulator combinations are referred to as “minimum function” mutations. For these patients, treatment with the next-generation CFTR corrector, elexacaftor, used in combination with tezacaftor and ivacaftor, known as Trikafta® (VertexGPS), has been found to show promising results in improving CFTR function and clinical outcome [62].

Importantly, CFTR protein function modulators have also shown promise in the treatment of CF-associated GI pathologies [63][64][65][66]. A key reagent for the preclinical testing of these drugs has been the development of patient-derived organoids from the colorectum [67][68]. The CFTR potentiator ivacaftor was found to modulate intestinal pH as well as the microbial population. Treatment of this compound resulted in the alteration of the gut microbiome and also reduced inflammation in patients with one copy of a CFTR gating mutation [69]. This effect on improving fecal microbiota via ivacaftor treatment was especially effective in patients with CF with pancreatic insufficiency [70].

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