The Clinical Biology of Cystic Fibrosis Transmembrane Regulator Protein

Its Role and Function in Extrapulmonary Disease

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Normal cystic fibrosis (CF) transmembrane regulator (CFTR) protein has multiple functions in health and disease. Many mutations in the *CFTR* gene produce abnormal or absent protein. CFTR protein dysfunction underlies the classic CF phenotype of progressive pulmonary and GI pathology but may underlie diseases not usually associated with CF. This review highlights selected extrapulmonary disease that may be associated with abnormal *CFTR*. Increasing survival in CF is associated with increasing incidence of diseases associated with aging. CFTR dysfunction in older individuals may have novel effects on glucose metabolism, control of insulin release, regulation of circadian rhythm, and cancer cell pathophysiology. In individuals who have cancers with acquired *CFTR* suppression, their tumors may more likely exhibit rapid expansion, epithelial-to-mesenchymal transformation, abnormally reduced apoptosis, and increased metastatic potential. The new modulators of *CFTR* protein synthesis could facilitate the additional exploration needed to better understand the unfolding clinical biology of *CFTR* in human disease, even as they revolutionize treatment of patients with CF.

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Biallelic mutations of the cystic fibrosis (CF) transmembrane regulator (*CFTR*) gene resulting in reduced or absent protein function cause CF.¹ The observed clinical syndrome of CF centers on airway disease characterized by inflammation, chronic infections, inspissation of tenacious secretions, bronchiectasis, and eventually premature death. This syndrome has been extensively reviewed.^{2,3} Beyond lung disease, CF involves multiple organs and

encompasses many clinical characteristics. Interactions between organ-specific disease processes within the clinical syndrome create a complex web of likely causal relationships with many possible positive feedback loops (Fig 1).^{2,4-8}

Increasing survival in CF allows identification of novel primary disease processes that may adversely affect glucose metabolism, control of insulin release, regulation of circadian

ABBREVIATIONS: AgRP = agouti-related peptide; ATP = adenosine triphosphate; CF = cystic fibrosis; CFRD = cystic fibrosis-related diabetes; CFTR = cystic fibrosis transmembrane regulator; NPY = neuropeptide Y

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Figure 1 – Causal inference diagram of cystic fibrosis clinical disease. Disease begins with allelic homozygosity of cystic fibrosis transmembrane regulator mutations and leads directly to abnormal secretions. Organ-specific disease ensues, directly affecting the lung, sinuses, pancreas, liver, and reproductive organs. Inflammation associated with airway infections and recurrent acute exacerbations of disease drive and are driven by worsening lung function.⁶ Factors intrinsic to patients such as age and sex alter the effects of cystic fibrosis on lung disease and survival.⁷ Malnutrition as a result of pancreatic and liver involvement affects the attainment of adult height and weight, and all three affect lung function and survival.^{7,8} Sinusitis and infertility do not lead to decreased survival but cause substantial worsening of quality of life, as do other manifestations of disease.^{2,4,5} Various complications of disease include diabetes, which contributes to early death. Treatments such as lung transplantation and liver transplantation markedly change disease and treatment profiles and have effects that alter survival.

rhythm, and cancer cell pathophysiology. Median observed survival has rapidly increased with new medications, treatments, and improvements in systematic care through rigorous accreditation processes by the Cystic Fibrosis Foundation in the United States,^{8,9} but survival is improving in the rest of the world, sometimes at an apparently faster rate with equally rigorous attention to the details of care.¹⁰⁻¹³ Older individuals are at greater risks of cognitive disorders, diabetes, sleep-wake disorders, cancer, and other chronic disorders.¹⁴⁻¹⁷ Discrimination between processes specifically related to CFTR dysfunction and those related to other processes associated with advanced age may enable design and implementation of effective strategies to treat these diseases in the setting of CF.

Treatments for CF have improved steadily since its description in English in 1938.¹⁸ The most recently added therapies, modulators of the gene product, augment biosynthesis and function of the CFTR protein, producing sometimes dramatic clinical improvements in patients with the appropriate targeted mutations.¹⁹ Because multiple diseases often coexist in aging individuals and may

increasingly appear in patients with CF, CFTR modulator therapy may become an increasingly important treatment with increasing age in CF.

The ability to directly address the underlying causes of progressive lung disease in patients with CF by using CFTR modulators may have unexpected but generally welcome effects on manifestations of abnormal *CFTR* in other organs. For patients without the classic clinical syndrome of CF, these same modulators may prove useful when *CFTR* mutations partially underlie pathophysiology. The present review examines the roles of *CFTR* and emerging data about the potential consequences of dysfunctional protein in multiple tissues and organs to explore some of the extent of *CFTR*-related disease. The new treatments have the potential to improve survival and quality of life in patients with CF as well as in others with more subtle or acquired defects in *CFTR* physiology.

These agents are intended to increase cell membrane CFTR protein activity. Corrector molecules increase CFTR protein delivery to the cell surface. Potentiators increase

anion flow through CFTR chloride channels already at the cell surface. Amplifiers improve translation of CFTR messenger RNA to increase CFTR protein production, ultimately to facilitate the action of correctors. Other agents that potentially increase CFTR protein activity do not clearly fit in these categories,²⁰⁻²³ or they have characteristics matching more than one category.

Cystic Fibrosis Transmembrane Regulator

The centrality of CFTR protein dysfunction in CF emphasizes its role in normal physiology. The CFTR protein is large, complex,²⁴ and responsible for multiple

functions that remain incompletely described. *CFTR* gene mutations are categorized according to the affected stage of protein production, activity, and disposal (Fig 2)^{2,3,25-27} and have recently been reviewed.^{2,3} In epithelial cells in the airway and elsewhere, CFTR protein transports chloride²⁸ and bicarbonate,^{26,29} and regulates other ion channels (namely sodium, potassium, other chloride and calcium channels).³⁰⁻³³ It also interacts with other membrane proteins to maintain epithelial tight-junctions and barriers to fluid flow.³³⁻³⁵ CFTR protein adjusts the levels of acidity in secretions that, along with fluid and chloride abnormalities, lead to

Figure 2 – Cystic fibrosis transmembrane regulator (CFTR) protein. CFTR protein consists of a 1480 amino acid chain.²⁵ Starting from the amine end, there is a Lasso motif, a membrane-spanning domain (amino acids [AA] 70-376) with an associated nucleotide-binding domain (AA 391-644), a regulatory domain (AA 654-838), another membrane-spanning domain (AA 841-1175), another nucleotide-binding domain (AA 1203-1437) and a final threonine-arginine-leucine tail at the carboxyl end. The two similar membrane-spanning domains work together to transport anions; the two similar nucleotide-binding domains mediate adenosine triphosphate hydrolysis, and opening and closing of the actual anion channel; and a threonine-arginine-leucine carboxyl terminal anchors the protein to the cytoskeleton and mediates interactions with other proteins in the cell. The three most common alleles are shown for most of the six categories of mutations. Arrows from each mutation name point to the approximate regions in the full protein that are affected in the underlying DNA sequence mutation as indicated by either the legacy amino acid or modern nucleotide-based mutation nomenclatures. The most common allele world-wide is F508del, a class II gene mutation. Mutation class is associated with degree of clinical severity, and common mutations occurring following the regulatory domain, AA 838, are associated with preservation of CFTR-associated bicarbonate transport and pancreatic sufficiency.²⁶ A regularly updated list of CFTR variants, allele frequencies, and disease-causing potential is available at www.cftr2.org, the source for the mutations noted in the figure.²⁷ Details regarding mutation classifications have been reviewed and are available as published.^{2,3}

some of the common manifestations of the CF clinical syndrome such as small airway disease and pancreatic insufficiency.^{26,29} CFTR participates in transport of sphingosine-1 phosphate protein,³⁶ a regulator of cell adhesion and a signaling molecule for inflammation.³⁷ It helps maintain antioxidant defenses by transporting glutathione and accounts for 45% of glutathione efflux from human bronchial epithelial cells.³⁸ At the subcellular level, CFTR maintains adequate internal acidification of several types of intracellular organelles,³⁹ thus maintaining their normal functions for a wide variety of intracellular processes.⁴⁰ The great variety of recently discovered cellular and intracellular activities suggests the potential for diverse manifestations of CFTR dysfunction.

Clinical Consequences of CFTR Dysfunction

Consequences of CFTR protein dysfunction are well known in the lung^{2,3} but may occur elsewhere. Pancreatic β -islet cell-related disease leads to CF-related diabetes (CFRD).⁴¹ Liver involvement frequently results in chronically elevated liver injury indicators in serum, and a minority of patients develop nonalcoholic steatohepatitis or cirrhosis, with a subset progressing to end-stage liver disease requiring transplantation.⁴² Abnormal secretions in the male reproductive tract lead to congenital absence of vas deferens and sterility; similar secretions in the female Fallopian tube impair passage of ova and sperm, reducing fertility.^{4,43} Tenacious secretions in nasal sinuses lead to chronic sinusitis in most patients with CF, often requiring daily medical treatment and occasional surgical debridement.⁵ Renal manifestations of CF include abnormal clearance rates for antibiotics used to treat lung infections.⁴⁴ Observed increased frequency of kidney stones seem to be due to unintended antibiotic-related alterations of the gut microbiome and increased systemic oxalic acid levels.^{45,46} Abnormal sweat, perhaps the oldest recognized manifestation of CF,^{28,47} leads to salt losses that may increase susceptibility to heat injury.⁴⁸

The Unfolding Clinical Expression of CFTR Pathophysiology

With improving survivorship, patients with CF become more likely to experience diseases associated with older age.⁴⁹ CFTR dysfunction may alter the expression of these disease states, create unusual presentations or possibly create unwelcome synergies to worsen age-related diseases.¹⁷ Cognitive dysfunction and diabetes related to aging are intertwined,⁵⁰ and CFTR

dysfunction may further complicate and increase morbidity. These disease processes, however, may provide additional treatment targets, especially with CFTR modulators, as well as new opportunities to measure clinically relevant outcomes that will require careful additional study. These modified presentations may provide insights for patients without CF experiencing similar disease states involving loss of CFTR function.⁵¹

CFTR and Modified Glucose Metabolism

Although the hypothesized core pathophysiology leading to CFRD is β -islet cell destruction during development of exocrine pancreatic insufficiency, some literature suggests that CFTR protein dysfunction directly causes glucose and insulin metabolic derangements that remain incompletely explored. In patients with CF, β -islet cells persist but exhibit morphologic and functional abnormalities.⁵²⁻⁵⁴ CFTR is normally expressed and active in pancreatic β -islet cells.⁵⁵ However, dysfunctional CFTR protein reduces insulin release from β -islet cells through multiple mechanisms that may include direct intracellular effects and autoinduced effects via abnormal secretion of IL-6.^{56,57} Failure of CFTR protein-mediated chloride transport alters membrane potential, reducing the ability of adenosine triphosphate (ATP)-sensitive potassium channels to depolarize the cell membrane in response to glucose,⁵⁸ which reduces cellular influx of calcium, an important step prior to insulin release.⁵⁹ Dysfunctional CFTR protein limits the function of other chloride channels via its regulatory activity, further limiting the effects of closure of ATP-sensitive potassium channels.⁵⁵ CFTR protein dysfunction seems to act in yet another way that compromises calcium binding to insulin granules, preventing insulin release itself.⁵⁶

CFTR protein dysfunction in the CNS⁶⁰ and GI tract⁶¹ affects glucose metabolism independently of exocrine pancreas effects. In addition to its recently reviewed CNS effects,⁶⁰ dysfunctional CFTR protein limits glucose-controlling mechanisms in the CNS. CFTR protein in glucose-inhibited neurons of the hypothalamus modulates release of neuropeptide Y (NPY) and agouti-related peptide (AgRP) in response to systemic glucose levels (Fig 3).^{53,60,62-66} In human neuronal cell models, hypoglycemia triggers an intracellular cascade of events leading to closure of the CFTR chloride channel, allowing secretion of AgRP.⁶⁵ Dysfunctional CFTR is associated with defective release of insulin-like growth factor in the pituitary, another

salt and fluid across the retinal pigment epithelium, which enables normal responses to light.⁷⁴ However, alcohol- and light-evoked electrooculograms in patients with F508del heterozygous or homozygous mutations were abnormal compared with non-CF control subjects,⁷⁵ most likely due to abnormal interactions between CFTR and other ion channels. The direct clinical consequence is unclear, but light reception is a critical step in normal entrainment of circadian rhythm within the hypothalamus.⁷¹ Measurement of electrooculograms in association with assessments of sleep-wake cycles may provide alternative methods of measuring the impact of treatment with CFTR modulators.

In animal models and humans, *CFTR* is expressed during embryonic brain development.⁶⁰ *CFTR* messenger RNA and protein are expressed in the hypothalamus, thalamus, and arcuate and amygdaloid nuclei, areas that control homeostasis and energy metabolism.^{60,62} *CFTR* messenger RNA expression occurs specifically in the anterior hypothalamus, the brain region in which the master circadian clock resides.⁷⁸

Disease manifestations linked to dysfunction in these areas of the brain include conditions associated with circadian rhythm and homeostasis perturbations such as insomnia, sleep disorders, mood and affect disorders, abnormal energy metabolism, growth, and reproduction.⁷⁸⁻⁸³ Many of these functions are difficult to discern as primary *CFTR* effects because of the sequelae of other features of CF, and they will require additional research to confirm. For example, although CF-associated female infertility could be related to neuron dysfunction leading to abnormal release of gonadotropin-releasing hormone as a primary effect of dysfunctional *CFTR*,⁸⁴ it may also be due secondarily to abnormal secretions in the reproductive tract and low body weight associated with pancreatic insufficiency and malnutrition.⁴

The pattern of neuronal involvement suggests the possibility of circadian rhythm disturbance mediated by abnormal *CFTR*. Entrainment or resetting of the master circadian clock in mammals requires the ability to sense and process light, which is disturbed by abnormal *CFTR*.^{71,74} Direct evidence shows that CFTR dysfunction compromises phase-shifting of the circadian rhythm,⁸⁵ which could prevent clinically normal responses to changing daylight hours, travel, or changing personal schedules to accommodate work or

school. A key function of daily circadian cycling is initiation of sleep. Acutely increased melatonin levels induce drowsiness and sleep, but neurons with deficient *CFTR* cannot respond normally to increased melatonin.⁸⁶ Abnormal responses in some people heterozygous for *CFTR* mutations⁷⁵ suggest that a far larger population may be affected. These findings predict that sleep phase may be delayed or otherwise altered in patients with CF, a conjecture consistent with recent clinical observations⁸⁷ but which will need confirmation and more detailed basic science support.

Sleep phase delay is associated with multiple adverse outcomes. Difficulty sleeping at conventional times, which may be diagnosed as insomnia,⁸⁸ can lead to chronic sleep deprivation because wake-up time is often fixed by morning responsibilities involving families, schools, and jobs. This form of circadian misalignment is similar to classic shift-work disorder⁸⁹ and may lead to academic failures^{90,91} and work-life difficulties.^{88,92} Depressive symptoms and anxiety are associated with circadian misalignment and sleep phase delay.^{80,93} Circadian disruption during pregnancy heightens the risk of adverse outcomes in humans.⁸³ Circadian rhythm misalignment increases the risks of obesity, diabetes, cardiovascular disease, and neural degeneration,^{16,81,82,94-97} and it may compromise normal immune function and tumor suppression.^{98,99} Many of these consequences overlap and potentially contribute to the extensive comorbidities of CF. A number of analyses explore disrupted sleep quality and latency in CF,¹⁰⁰⁻¹⁰³ but sleep phase delay remains relatively unexplored.⁸⁷ The potential for adverse outcomes suggests that studies in CF centered on sleep phase and circadian misalignment may find high-yielding, novel treatment opportunities for *CFTR* modulators.

CFTR and Cancer

Many cancer types rise in incidence with advancing age,¹⁵ and patients with CF are reaching older ages in rapidly growing numbers.⁸ Systematic investigations show that patients with CF have an increased risk of cancer.¹⁰⁴ The majority of observed tumors originate in the GI tract, particularly from the ascending colon, but include gallbladder and small intestine tumors that occur uncommonly or rarely in the general population. Outside of the GI tract, lymphoid leukemia and testicular carcinomas occur more frequently than expected while the incidence of melanoma seems significantly decreased, possibly because patients with CF avoid sunlight to prevent heat injury.⁴⁸

Genetic and Acquired Abnormal *CFTR* Functions in Cancer Cells

Observations reporting increased cancers in patients with CF cannot distinguish between a secondary effect of CF-associated chronic inflammation as seen in the colon or a primary effect of *CFTR* dysfunction. For example, intestinal-specific *CFTR* knockout mice had greatly increased development of colon and small intestine tumors compared with wild-type mice; however, the mouse GI tract had increased inflammation and dysregulated inflammatory response pathways.¹⁰⁵ Nevertheless, investigations of human samples and cancer cell lines suggest that acquired *CFTR* dysfunction increases cancer aggressiveness, morbidity, and mortality independently of inflammation.¹⁰⁶⁻¹⁰⁸

Among patients with CF, tumors of the lung are too few to ascertain a difference in occurrence rate compared with the general population.¹⁰⁴ However, *CFTR* suppression occurs commonly among lung tumors in patients without CF. Hypermethylation of the *CFTR* promoter region affected 41.7% of squamous cell cancers and 21.5% of adenocarcinomas in a study of 139 patients with lung cancer.¹⁰⁸ Specific *CFTR* sequence mutations occurred in four of 17 patients with non-small cell lung cancer¹⁰⁹ and in four of 22 patients with unspecified lung cancers.¹¹⁰

CFTR suppression may enhance deleterious cancer cell behaviors. In vitro studies of lung cancer cell lines revealed decreased adhesion, increased epithelial-to-mesenchymal transition, motility, and invasiveness of *CFTR*-deficient cells.¹¹¹ Carefully controlled experiments with murine cancer models revealed more rapid tumor growth and increased metastases that were attenuated by increasing *CFTR* expression.^{105,111} Clinically, patients with tumors with decreased *CFTR* expression presented with more advanced lung cancer stages. After correction for staging, these patients had decreased survival relative to patients with tumors with normal *CFTR*.^{108,111} Although these findings suggest that *CFTR* may be more than a passenger gene in lung cancer, they require confirmation.

Other cancer types exhibit heterogeneous expression and function of *CFTR* protein. For example, in prostate cancer cell lines, *CFTR* suppression was associated with decreased adhesion, increased cell growth, and increased migration, findings that were reversed with restoration of *CFTR* function.¹⁰⁷ *CFTR* suppression by hypermethylation of the promoter region occurred in 47% of prostate adenocarcinomas from 83 patients.¹¹²

These patients had decreased disease-free survival and decreased overall survival compared with those without *CFTR*-suppressed tumors. The frequency of sequence mutations in prostate cancer has not been explored.

Conversely, there is early evidence that *CFTR* suppression may improve some cancer treatment outcomes. *CFTR* is strongly associated with cyclic guanosine monophosphate-protein dependent kinase I in enrichment networks of tumor spheres for two glioma cell lines. Further analysis tentatively suggests that suppression of these associated genes may be helpful in the treatment of glioblastoma.¹¹³ Other early evidence suggests that *CFTR* overexpression may be associated with increased aggressiveness of ovarian cancer,¹¹⁴ a finding complementary to the suggestion of increased testicular cancer in people with CF.¹⁰⁴

Unfortunately, no investigation has yet found a mechanism directly linking abnormal *CFTR* gene expression with modified cancer incidence or aggressiveness. Associations between *CFTR* protein and recognized cancer markers may generate plausible hypotheses suitable for testing (Table 1).^{35,106,107,113,115-125}

Treatment With *CFTR* Modulators

Several approved *CFTR* modulators slow lung disease and reduce pulmonary exacerbation frequency in CF.¹⁹ However, the multisystem adverse effects of abnormal *CFTR* suggest that modulators may have other positive therapeutic outcomes. Some CF manifestations do not lead to rapid demise and thus have not been fully assessed as primary or even secondary outcomes in clinical trials.

CFTR modulators may prevent or delay the onset of CFRD in patients with treatable mutations by repairing *CFTR* function.¹²⁶ Reduced *CFTR* dysfunction might improve associated β -islet cell dysfunction.^{55,56,60,127} *CFTR* modulators could improve CNS regulation of glucose metabolism^{65,67} depending on ability to reach glucose-sensing neurons.

CFTR modulators may also improve olfaction and light sensation. Restoration of olfaction has the potential to improve appetite, mood, and quality of life.¹²⁸ Normalized processing of light signals coupled with improved *CFTR* protein function in the anterior hypothalamus might improve master circadian clock entrainment, a change that could have widespread positive effects. For example, improvement of *CFTR*

TABLE 1] Potential Involvement of CFTR With Other Proteins Implicated in Cancer

Target Protein	Known Relationships With <i>CFTR</i>	Effect of <i>CFTR</i> Dysfunction	Potential Tumor Effects
AF-6/ afadin	Colocalizes at cell-cell contacts and physically interacts with <i>CFTR</i> ³⁵	Reduces epithelial tightness; reduces afadin ³⁵	Increases metastatic potential ³⁵
EGFR	<i>CFTR</i> suppresses EGFR signaling and IL-8 production ¹¹⁵	Activates EGFR pathway involving IL-1 α and TNF- α converting enzyme ¹¹⁵	EGFR enhances VEGF effects in cancer ¹¹⁶ Accelerates cell proliferation and tumor growth ¹¹⁷
p38 MAPK	<i>CFTR</i> inhibition increases p38 MAPK phosphorylation ^{118,119}	Increases p38 MAPK activity leading to increased inflammatory cytokines ¹²⁰	Increases epithelial-to-mesenchymal transformation ¹²¹ Decreases apoptosis ¹²²
cGMP- PRKG1	Strong linkage with <i>CFTR</i> in network analysis ¹¹³	May decrease PRKG1 activity ¹¹³	Decreased PRKG1 may reduce tumor growth ¹¹³
uPA	Suppresses uPA activity via suppression of NF κ B ^{106,107}	Allows upregulation of NF κ B and uPA activities	Increases epithelial-to-mesenchymal transformation ¹⁰⁶ Increases metastatic potential ¹⁰⁶
VEGF	Elevated in sputum and serum with pulmonary exacerbations of CF ^{123–125}	Induces VEGF synthesis ¹²⁴	Enhances angiogenesis and tumor growth ¹¹⁶

CF = cystic fibrosis; *CFTR* = cystic fibrosis transmembrane regulator; cGMP = cyclic guanosine monophosphate; EGFR = epidermal growth factor receptor; MAPK = mitogen-activated protein kinase; PRKG1 = protein-dependent kinase I; TNF- α = tumor necrosis factor α ; uPA = urokinase plasminogen activator; VEGF = vascular endothelial growth factor.

functioning may decrease phase delay in sleep⁸⁷ by shifting the increase in circulating melatonin closer to the end of the astronomical day and improving the response to melatonin to facilitate the onset of biological night.

In cancers in which acquisition of *CFTR* suppression seems to augment the severity and progression of the disease, modulating agents may improve outcomes. For example, in prostate cancer, reduction in aggression in some tumors may obviate invasive treatments, reducing costs and morbidities and improving quality of life and survival. However, caution is needed as there are other cell types in which the early data suggest that increased *CFTR* activity increases tumor aggressiveness.^{113,114}

Because *CFTR* suppression may be due to hypermethylation of the gene promoter or any one of a large number of sequence mutations, many different approaches will be needed to realize the potential benefits of modulator treatments. *CFTR* modulators may be useful in novel sequence mutations found in cancer cells for which knowledge of isolated *CFTR* dysfunctional effects are necessarily limited. Hypermethylation seems to be a common mechanism for *CFTR* suppression but might still allow synthesis of a

certain number of protein molecules, and agents that improve trafficking and stability of F508del *CFTR* protein and slow scavenging of old proteins^{20,51} could have a positive impact.

All the putative applications of *CFTR* modulators mentioned here and the potential effects must be more carefully defined than the present discussion. Carefully crafted new hypotheses must then undergo rigorous testing prior to deployment of these medications for novel, additional purposes.

Conclusions

The treatment of CF has been successful enough to allow the characterization of multiple manifestations of *CFTR* dysfunction beyond progressive airway and pancreas destruction. Glucose and insulin metabolism abnormalities due to abnormal *CFTR* seem to partially underlie malnutrition, poor growth, and CFRD; discoveries of the role of *CFTR* protein in these processes could lead to insights relevant to better understanding and possibly treatment of obesity and diabetes in people without CF. *CFTR* dysfunction in the nervous system may cause disordered energy homeostasis, abnormal pain perception, dysregulation of reproduction, and decreased

plasticity of circadian rhythm entrainment. This finding in turn has implications for immune function, sleep phase, mood abnormalities, and cancer incidence. Successful studies in cancer biology have shown that frequently occurring *CFTR* suppression leads to cell behaviors that predict increased tumor growth, rapid metastasis, and earlier mortality. The progress in treatment of CF and the discoveries of *CFTR* involvement in the multiple cell types involved in a wide array of conditions suggest multiple new avenues for investigation at basic through clinical levels. The ongoing development of *CFTR* modulators promises to facilitate many of these studies and assist the design and implementation of novel treatment strategies for a diverse array of human diseases.

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