

Altered Mitochondrial Function and Cystic Fibrosis

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Abstract

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutation in cystic fibrosis transmembrane conductance regulator gene (CFTR). CFTR is a membrane glycoprotein which functions as cyclic adenosine monophosphate (cAMP) activated anion channel. Mutation in CFTR effect's its synthesis, processing, regulation and function resulting in CF. Mutated CFTR results in dysregulation of fluid and electrolyte transport across epithelial cell membranes. Mutated CFTR has also been implicated to alter mitochondrial structure and function. All the probable mitochondrial functions like components of electron transport chain, calcium buffering, reactive oxygen species generation, levels of mitochondrial glutathione have been implicated to be effected in CF. CFTR has also been reported to regulate functioning of two mitochondrial genes C1SD1 and MT-ND4. All the reports illustrating the role of CF in altering mitochondrial function are scrutinized and presented in this review.

Keywords: Cystic fibrosis; Mitochondria; CFTR; MT-ND4; C1SD1; Calcium; Glutathione

Abbreviations: CF: Cystic Fibrosis; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; ABC: ATP-Binding Cassette; MSD: Membrane Spanning Domains; NBD: Nuclear Binding Domain; GSH: Glutathione Stimulating Hormone; GR: Glutathione Reductase; ROS: Reactive Oxygen Species

Introduction

Cystic fibrosis (CF) is an autosomal recessive multisystem disorder [1,2]. It is caused by mutation in cystic fibrosis transmembrane conductance regulator gene (CFTR) [1-3]. CFTR is a membrane protein which acts a channel for transport of chloride ions [3]. Transport of chloride ions regulate water and salt content of the epithelial surface resulting in thin mucus formation, predominantly in respiratory, digestive and reproductive tissues [1-5]. Disruption of ion transport due to mutated CFTR gene results in production of thick viscous mucus and increased salt concentration in the sweat (Figure 1) [1-5]. It results in blockage of the airways, ducts, glands resulting in complications like pulmonary failure, gastrointestinal disorders as well as infertility [1-6].

CFTR is a 170 kDa membrane glycoprotein belonging to ATP-binding cassette (ABC) transporter gene super family [7]. CFTR protein has two membrane spanning domains (MSD), made up of six helices each which are connected to the nuclear binding domain (NBD 1 and

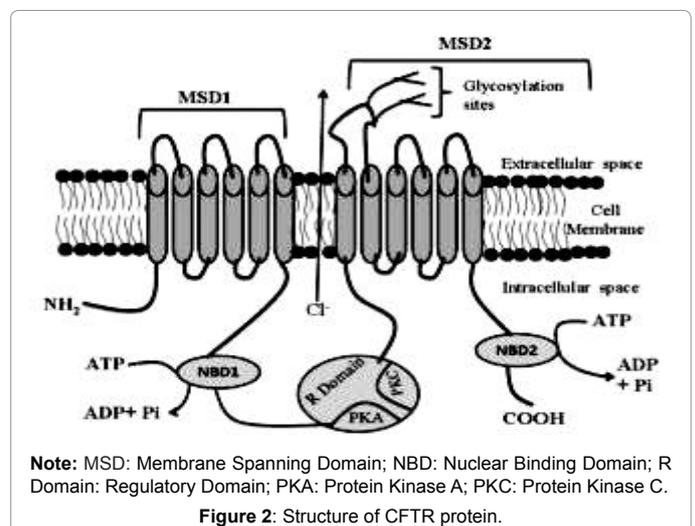
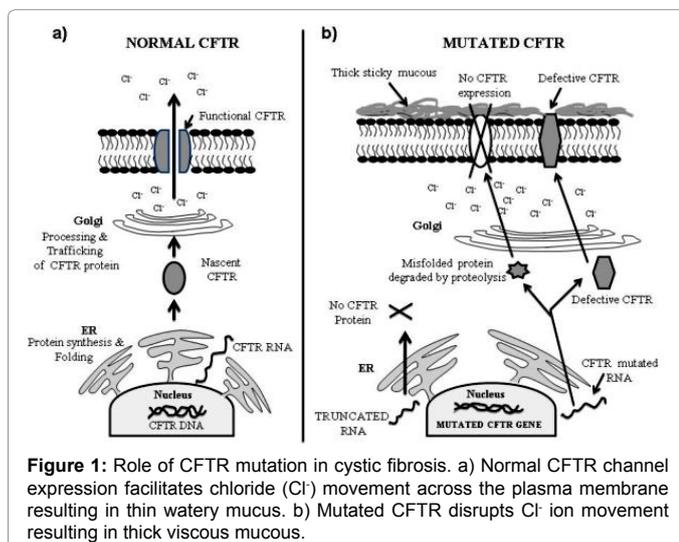


Figure 2: Structure of CFTR protein.

2). NBD's are involved in ATP binding and hydrolysis [7]. In between NBD 1 and MSD2 is present regulatory (R) domain. The R domain contain sites for cyclic adenosine monophosphate (cAMP) dependent phosphorylation substrates for protein kinase A and C. ATP binding to NBD and phosphorylation of R domain regulates ion channel activity of CFTR (Figure 2) [7]. Around 1997 CFTR gene mutations are listed in the cystic fibrosis mutation database (<http://www.genet.sickkids.on.ca>). Deletion of phenylalanine at position 508 (F508del-CFTR), is the most predominant mutation reported to be associated with CF and it is present in NBD 1 [8].

Mutated CFTR causes defective intracellular transport resulting



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in CF etiology. Role of defective CFTR in altering mitochondrial functioning has been reported. All the available literature illustrating alteration of mitochondrial functioning in cystic fibrosis has been reviewed and accounted in the underlying sections.

Mitochondrial function and cystic fibrosis: Evidence in 1970's and 1980's

The role of altered mitochondrial function in CF was hypothesised as early in 1979 by Burton L. Shapiro et al. [9-13]. Using cultured fibroblast cells from the CF patients and control they have reported that CF cells showed more oxygen consumption compared to the control, moreover inhibition of the mitochondrial electron transport system using rotenone resulted in distinctive rates of inhibition of oxygen consumption in control and CF cells [10]. Nicotinamide adenine dinucleotide (NADH) dehydrogenase, which is the enzyme of complex I of the mitochondrial electron transport system also showed differences in enzyme kinetics with decreased Km and increased pH optima in CF cells [9-14]. Similarly, altered kinetics for cytochrome-c oxidase resulting in increased Km at temperature >25°C have been reported in CF fibroblast [15].

CF cells were also reported to show increase in the intracellular calcium concentration compared to control [16-18], moreover mitochondria showed enhanced calcium uptake [18]. They have also reported an increase in relative electron transport activity of mitochondrial complex in case of NADH-oxidase, NADH-cytochrome c reductase, and succinate-cytochrome c reductase in CF cells compared to control [18]. Altered levels of glutathione (GSH) and activity of glutathione reductase (GR) has been reported in CF compared to control [19,20]. Altered levels of GSH are implicated in the conditions of oxidative stress, moreover, mitochondria are one of the prime sites for oxidative stress and GSH plays a critical role in maintaining redox homeostasis. All these lines of evidences reported in late 1970's and 1980's supported the role of mitochondrial dysfunction in CF.

Mitochondrial dysfunction and cystic fibrosis: Post 1990's

Advent of cloning technique facilitated cloning of CF gene in late 1980's and revealed it as a chloride channel [3,4]. Further research facilitated that CFTR facilitates expression of several genes. CFTR has been reported to regulate expression of RANTES which are chemokines mediating mucosal immunity [21]. Similarly, CFTR has also been reported to regulate MUC1 (mucins) and tyrosine kinase c-Src expression [22]. Role of mitochondrial dysfunction in CFTR became more confirmative by the finding of CFTR dependent regulation of two mitochondrial genes CISD1 and MT-ND4 [23,24]. CISD1 gene encodes for a protein with a CDGSH iron-sulfur domain and which is localized to the outer mitochondrial membrane whereas, MT-ND4 encodes for MT-ND4 mitochondrial Complex I (mtCx-I) subunit [23,24]. Down regulation of CISD1 has been reported in CF cells, similar results were reported when CFTR chloride channel activity was disrupted using chemical inhibitors [23]. Similar results of down regulation of MT-ND4 in CF cells or an inhibition of CFTR chloride transport have been reported [24].

Role of CFTR in regulating MT-ND4 also supports the initial observation of Shapiro et al regarding altered kinetics of NADH dehydrogenase enzyme of mitochondrial complex I [11,13]. MT-ND4 also known as mitochondrially encoded NADH dehydrogenase 4 encodes for NADH dehydrogenase 4 protein (ND4), which is one of the subunit of NADH dehydrogenase enzyme or complex I of mitochondrial respiratory chain [24]. In concurrence with the observation of down regulation of MT-ND4 gene expression in CF

cells, reduction in mitochondrial complex I activity as well as altered mitochondrial membrane potential has been demonstrated [25,26].

CISD1 proteins also named as mitoNEET have been reported to belong to the family of iron-sulfur (2Fe-2S) proteins with Fe-S domain comprising of CDGSH amino acid sequence [27-29]. mitoNEET proteins have implicated in regulating cellular iron and homeostasis of reactive oxygen species (ROS) [27-29]. Suppression of mitoNEET proteins has implicated to result in accumulation of iron, ROS and autophagy [27]. However role of CISD1 downregulation in CF still remain unclear, but it may be due altered mitochondrial function due to accumulated iron and ROS.

Another important function of mitochondria is Ca²⁺ buffering to regulate Ca²⁺ signalling. Ca²⁺ signalling is vital for many cellular functions and deregulation of it has been reported in CF. Deregulation of mitochondrial Ca²⁺ buffering resulting in increased Ca²⁺ accumulation compared to control has been reported in mitochondria obtained by fibroblast culture from CF patient in research reported in 1970's and 1980's [16-18]. A study done using F508del-CFTR airway epithelial cells have reported reduced Ca²⁺ levels in CF cells compared to control [26]. They have also reported alteration in mitochondrial morphology to more fragmented form in CF human tracheal gland cells compared to control [26]. Mitochondrial membrane potential has also been reported to be reduced in CF cells compared to control [26]. Another study published recently has reported role of F508del-CFTR accumulation in endoplasmic reticulum (ER) in deregulation of Ca²⁺ signalling in CF [30]. They have reported that accumulation of F508del-CFTR in ER results in increased SERCA (Sarcoendoplasmic Reticulum Ca²⁺ Transport ATPase) pump activity on the other hand activity of PMCA (Plasma Membrane Ca²⁺ ATPase) gets reduced [30]. The possible role of increased CFTR/SERCA interaction and decreased CFTR/PMCA in CF cells in deregulation of Ca²⁺ homeostasis has been reported [30]. Increased Ca²⁺ retention in ER was reported due to F508del-CFTR accumulation and the effect was rescued following F508del-CFTR clearance [30]. Mitochondrial Ca²⁺ uptake has also been reported to increase in CF cells, however clearance of F508del-CFTR ER trafficking failed to restore normal mitochondrial Ca²⁺ levels [30].

Apart from transport of chloride ions role of CFTR in GSH transport has also been reported [31-33]. Altered GSH level in CF has been reported by the initial studies done in 1970's [19,20]. They have demonstrated higher, GSH reductase activity and total GSH levels in blood cells of CF patients compared to non CF [19]. Studies done during 1990's demonstrated GSH deficiency in CF samples. Decreased GSH levels have been reported in CF epithelial lining fluid (ELF), apical medium obtained from CFTR-deficient cell culture [32,34] moreover, transfection of normal CFTR has been reported to result in increased GSH [32]. On the similar line study done using CFTR knockout (CFTR-KO) mice demonstrated decreased GSH levels in ELF, whereas activity of glutathione reductase and glutathione peroxidase were elevated in CFTR-KO lung tissue [33,35].

Oxidative stress due to defective CFTR together with dysregulation of antioxidative effect due to decreased GSH level results in CF disease progression and its pathological hallmarks. Mitochondrial electron transport system is one of the major sources of ROS generation [36] and GSH plays an antioxidative role. Mitochondria do not produce GSH and thus are dependent on GSH uptake from the cytosol. Decreased levels of mitochondrial GSH have been reported in the lungs of CFTR-KO mice and CFTR-deficient human lung epithelial cells; mitochondrial oxidative stress was also increased [33]. Another study reports depleted mitochondrial GSH levels in CF impairs functioning

of electron transport chain (complex I) and restoring of GSH levels by GSH monoethyl ester (GSH-EE) restores electron transport chain functioning and mitochondrial membrane potential [35]. GSH-EE was also reported to re-establish normal levels of Interleukin-8 (IL8) in CF cells [37].

Conclusion

A mutation in the CFTR protein hampers its biosynthetic processing resulting in its proteolytic degradation in ER [38]. Proteolytic degradation of CFTR results in disrupted intracellular ion transport, oxidative stress, imbalance in redox homeostasis and resulting in pathological features of the disease like increased sodium and chloride levels in sweat, viscous mucous secretion, infection, inflammation etc. Mutated CFTR protein has been reported to affect mitochondrial structure as well as function. CFTR has also been reported to regulate expression of few mitochondrial genes. Functioning of mitochondrial electron transport chain, Ca^{2+} homeostasis, redox balance, GSH levels all have been reported to be distressed in CF. Thus further exploration of mechanism underlying regulation of mitochondrial structure and function by CFTR gene may open possible avenues for CF therapeutics.

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