Chronic Fatigue and Immune Deficiency Syndrome (CFIDS), cellular metabolism, and ionizing radiation: A review of contemporary scientific literature and suggested directions for future research

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To cite this article: Andrej Rusin, Colin Seymour & Carmel Mothersill (2018): Chronic Fatigue and Immune Deficiency Syndrome (CFIDS), cellular metabolism, and ionizing radiation: A review of contemporary scientific literature and suggested directions for future research, International Journal of Radiation Biology, DOI: 10.1080/09553002.2018.1422871

To link to this article: https://doi.org/10.1080/09553002.2018.1422871
Short Title: Ionizing radiation effects on CFIDS, cell metabolism

REVIEW

Chronic Fatigue and Immune Deficiency Syndrome (CFIDS), cellular metabolism, and ionizing radiation: A review of contemporary scientific literature and suggested directions for future research

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Supplemental Material for this article can be accessed on the publisher’s website.

Abstract

Purpose: To investigate biochemical pathways known to be involved in radiation response and in CFIDS to determine if there might be common underlying mechanisms leading to symptoms experienced by those accidentally or deliberately exposed to radiation and those suffering from CFIDS. If such a link were established, to suggest testable hypotheses to investigate the mechanisms with the aim of identifying new therapeutic targets.

Conclusions: Evidence for involvement of the alpha-synuclein, cytochrome c oxidase, αB-crystallin, RNase L, and lactate dehydrogenase/STAT1 pathways is strong and suggests a common underlying mechanism involving mitochondrial dysfunction mediated by ROS and disruption of ATP production. The downstream effect of this is compromised energy production. Testable hypotheses are suggested to investigate the involvement of these pathways further.

Key Words: Chronic Fatigue Syndrome, Bystander effects of radiation, Reactive oxygen species (ROS), Post-Radiation Syndrome, Atomic Veterans
Introduction

Atomic Veterans and Chronic Fatigue

Victims of radiation accidents such as Chernobyl or Fukushima, and those exposed deliberately in the Atomic Bombs, the Cold War nuclear detonations, and the more recent Gulf War complain of diffuse symptoms many years later (Loganovsky 2000; Milano 2000; Durakoviæ 2003; Bertell 2006; Broinowski 2013). Generally, their contention that ionizing radiation exposure led to their symptoms manifesting up to 60 years later are dismissed as non-causal due to the allegedly low doses they received and the lack of sufficient numbers to establish good epidemiology (McCauley et al. 2002; Coughlin et al. 2013; Mothersill and Seymour 2016).

Many of the symptoms and disease manifestations are similar to those seen in patients diagnosed with Chronic Fatigue and Immune Deficiency Syndrome (CFIDS).

The onset of symptoms following acute radiation exposure has been termed Post-Radiation Syndrome (PRS), an illness studied mainly in atomic bomb and Chernobyl survivors. A few studies have noted that the pathology of PRS is very similar to a family of multisystem illnesses including CFIDS, fibromyalgia, and multiple chemical sensitivity, according to Pall (2008). This connection was mainly made due to a similar onset of the disease after a short-term stressor, the same chemical markers appearing in both illnesses, and similar symptoms (Loganovsky 2000; Pall and Satterlee 2001; Pall 2007, 2008, 2009). According to the review published by Pall (2008), while the pathways thought to be involved in PRS were mostly studied in patients exposed to very high doses of radiation, other studies have demonstrated that much lower doses can result in activation of the same pathways (Mohan and Meltz 1994; Prasad et al. 1994;
Yin et al. 2003; Mitra et al. 2005; Rithidech et al. 2005). These include doses within the range experienced by the Chernobyl Liquidators (Kumerova et al. 2000). The same study discussing Chernobyl Liquidators indicated that the workers experienced symptoms consistent with CFIDS, although it did not mention the disease by name. There have been many studies that have linked low-dose radiation exposure to CFIDS directly, primarily due to neurophysiologic and metabolic changes that occur in some patients; studies include those conducted on Chernobyl survivors and Gulf War veterans. Among these are studies showing effects in patients receiving total biological doses of 0-70 mSv (Pflugbeil et al. 2006; Pall 2008; Loganovsky 2009; Bazyka et al. 2010; Loganovsky et al. 2016).

While no clinical study has directly linked CFIDS with radiation exposure, some symptoms documented in victims of radiation accidents and in radiotherapy patients were shown to be consistent with symptoms of CFIDS, including fatigue, immune system dysfunction, and cognitive dysfunction (Pastel et al. 2002; Pflugbel et al. 2006; Pall 2008; Loganovsky 2016; Mothersill and Seymour 2016). Such symptoms are often dismissed as inconsequent to radiation exposure or as radiophobia mainly on the grounds that they do not appear to be dose dependent and are reported by people who have been exposed to doses considered too low to cause biological effects (Pastel 2002; Mothersill and Seymour 2016). What these authors are ignoring is that non-targeted effects of radiation (NTE) have an exceedingly low threshold for induction in the region of 2-5mGy (Liu Z et al. 2007; Schettino et al. 2005) and once turned on they continue to be expressed over time, including over generations (Nagasawa and Little 1992; Mothersill and Seymour 1997; Wright 1998; Limoli et al. 2000; Mothersill et al. 2000; Morgan 2003; Smith et al. 2007; Lorimore et al. 2008; reviewed in Nugent et al. 2010; Shi et al. 2016;
Smith et al. 2016). The second point about NTE is that the effects saturate at relatively low doses, being fully expressed after a 0.5 Gy acute dose of gamma radiation (Seymour and Mothersill 2000; Liu Z et al 2007). Therefore, it is not surprising that there is no correlation of these symptoms with dose; this idea has been discussed and reviewed in Mothersill and Seymour 2016 cited above.

**Chronic Fatigue and Immune Deficiency Syndrome**

CFIDS is a disease without a definitive cause or mechanism. CFIDS patients claim that low dose exposure to ionizing radiation may be a common factor involved in their disease (Loganovsky 2000; Pastel 2002). While investigation into the disease has yielded many findings over the last few decades, the full nature of the disease remains unresolved. The purpose of this review is to examine the literature concerning a few core biochemical pathways, such as mitochondrial dysfunction, the cellular oxidative stress response, and inflammatory processes, where radiation exposure is known to cause perturbations and where there is evidence that the pathway is aberrant in patients with CFIDS. By drawing the two largely independent fields of CFIDS and radiation research together, it is hoped that common ground can be found which could inform new testable hypotheses in the area.

CFIDS is a disease primarily characterized by persistent or recurring long-term fatigue and susceptibility to infections (Holmes et al. 1988). Patients present a combination of other symptoms in addition to the ubiquitous lassitude, including headache, muscle aches, impairment of neurocognitive functions, and reduced quality of sleep (Evengård et al. 1999),
symptoms usually encountered in patients following acute radiation exposure as well
(Kumerova et al. 2000; Pastel 2002). Considering that the disease has affected millions since it
was discovered, it is surprising that researchers are still uncertain about the exact cause of
CFIDS and the mechanisms behind its manifestation. Despite some stigma towards the pursuit
of research concerning CIFDS outside of a psychopathology (Anderson et al. 2013), recent
studies have shown that CFIDS almost certainly has a physiological basis. While it is important
to distinguish between the putative immunologic, physiologic, and neurologic models of CFIDS,
it is also important to understand, as eloquently argued by Whiteside & Friberg (1998), the
potential for interconnectedness among the three and work towards developing a
comprehensive model that explains all facets of the illness. Among the potential immunologic
and neurologic factors that may contribute to the onset and maintenance of CFIDS pathology,
one of the most exciting areas involves the investigation of the metabolic symptoms of the
disease.

CFIDS, Mitochondria, and Metabolism

A recent comprehensive study of the metabolomics of patients with CFIDS carried out by Dr.
Robert Naviaux et al. (2016) effectively showed that the disease can be characterized as a
“highly concerted hypometabolic response” to environmental stress, and one that can be easily
traced to mitochondria. Specifically, the study showed that the metabolic response was
homogeneous to heterogeneous stressors by examining a variety of biochemical pathways that
were consistently altered or abnormal in patients with CFIDS. The study also suggested that
every metabolic abnormality observed in CFIDS patients was either regulated directly by redox reactions or the availability of NADPH. In addition to this, they found that many of the NADPH-dependent enzymes identified in the study had mitochondrial isoforms that were known to be upregulated during periods of environmental stress. They describe NADPH as a “global barometer of cellular fuel status” by interacting with both mitochondrial NADH consumption and the availability of similar reducing agents in the cytosol. Considering the findings of this paper, it is not only appropriate to propose that CFIDS may be intrinsically linked to metabolic dysfunction, but also that it may be consequently inextricably linked to the function, or rather the dysfunction, of mitochondria.

The idea that CFIDS is correlated to the improper functioning of mitochondria is not a radically new one; in fact, it has been described by many authors in the past. When taking into account the symptoms of CFIDS alone, they all appear to stand out as a hallmark of mitochondrial dysfunction in terms of energy production and deficit in the cell (Filler et al. 2014). The same review by Filler et al. (2014) proposed that significant downregulation of genes associated with antioxidant mechanisms, aerobic energy production, and metabolism in peripheral blood mononuclear cells of CFIDS patients when compared to control groups. The article also described proper mitochondrial function as negatively correlated with fatigue symptoms and another study determining the partial blockage of an ADP-ATP translocator in the mitochondria in patients with CFIDS (Booth et al. 2012). Oxidative stress, which can be caused by the presence of compounds such as radical oxygen species (ROS) and radical nitrogen species (RNS) in a cell, is another feature indicative of mitochondrial dysfunction (Lin & Beal 2006) which is also observed in patients with CFIDS, according to the literature. One study (R Kurup & P Kurup
2003) showed that antioxidants, which include compounds such as superoxide dismutase that are produced by cells to combat oxidative stress, were decreased in patients with CFIDS; in addition to this, they suggested that, due to decreased free-radical scavenging, that ROS and RNS were increased in plasma samples of CFIDS patients when compared to control groups potentially leading to mitochondrial dysfunction. They proposed that free-radical generation could be implicated in the pathogenesis of CFIDS, accounting for muscle pain and fatigability in patients. ROS have been shown to be involved in a variety of diseases and therefore their presence generally results in a greatly diminished quality of life if improperly regulated in human cells; high concentrations of ROS in a cell can cause oxidative damage to integral metabolic components (Panieri & Santoro 2016), DNA damage (Tada-Oikawa et al. 2000), and can eventually induce apoptosis (Armeni et al. 2004). Interestingly, one clinical study (Fulle et al. 2000) noted specific oxidative modifications and reduced oxidative metabolism in the myocytes of patients with CFIDS. Another study (Wawrzyniak et al. 2016) showed impaired biogenesis signalling and abnormal mitochondrial content in the myocytes of older individuals with idiopathic, or rapid-onset, chronic fatigue. When taken as a whole, these findings suggest that oxidative muscle damage and impaired mitochondrial function could be culprits in the onset of CFIDS. Due to the presence of mitochondrial dysfunction in patients with CFIDS and organelle’s tendency to produce damaging radical chemical species (RCS) if perturbed, the mechanisms that contribute to oxidative stress and their relationship to the disease should be of particular interest to researchers.

According to a study by Myhill et al. (2009), there is considerable evidence that mitochondrial dysfunction is present in some sufferers of CFIDS. Biopsies of human muscles in CFIDS patients
have shown abnormal mitochondrial degeneration. (Byrne et al. 1985; Behan et al. 1991; Vecchiet et al. 1996). Further studies have shown that CFIDS patients contain deletions in mtDNA genes associated with energy production, specifically in skeletal muscle (Zhang et al. 1995; Vecchiet et al. 1996). Increased activity of antioxidant enzymes and RCS-linked oxidative damage has been observed in the muscles of CFIDS patients (Fulle et al. 2000). Decreased levels of chemicals essential in metabolic reactions in mitochondria have also been shown to be deceased in CFIDS patients. (Kuratsune et al. 1994; Plioplys and Plioplys 1995). Reduced oxidative metabolism has also been observed in patients with CFIDS along with higher levels of lactate and pyruvate (Arnold et al. 1984; Buist 1989; Wong et al. 1992; McCully et al. 1996; Lane et al. 1998). Two studies showed that lipid replacement and antioxidant nutritional therapy administered through a dietary regimen can help restore mitochondrial function and alleviate fatigue symptoms (Nicolson 2003; Nicolson and Ellithorpe 2006).

The Evidence for Potential Involvement of Radiation

Many publications have linked ionizing radiation to the mitochondria-dependent generation of ROS and RNS, primarily linking it to radiation-induced bystander effects and genomic instability (reviewed in Szumiel 2015). According to Azzam et al. 2012, short and long-term radiation induced ROS/RNS could result to damage to mitochondrial DNA in genes coding for electron-transport subunits and their associated proteins. Cells without a functioning electron transport chain do not experience ROS/RNS production (Leach et al. 2001). Cells with ROS/RNS induced mtDNA damage could produce dysfunctional proteins associated with the electron transport
chain, resulting in pro-oxidant chemical formation, which can eventually lead to de-novo oxidative damage to biological structures long after radiation exposure with the mtDNA potentially being passed on to daughter cells after division contributing to genomic instability (Bogenhagen and Clayton 1977; Spitz et al. 2004; Gaziev and Shaïkhaev 2007; Azzam et al. 2012). An in-vivo study using mice exposed to 0.2 Gy of x-rays found significant changes in pyruvate metabolism and structural proteins persisting four weeks after exposure in heart cells. Higher doses resulted in respiratory complex I and III partial deactivation as well (Barjaktarovic et al. 2011). A few studies have shown that mitochondrial protein import could be used as a marker for the long-term effects of ionizing radiation exposure, even at small doses, as this could be the result of damage to components in translocational machinery or changes to membrane potential (Pandey et al. 2006; Azzam et al. 2012).

After reviewing the literature, it is plausible to suggest that the production of ROS due an endogenous or exogenous cause can contribute to the dysfunction of mitochondria in patients suffering from CFIDS, among other potential causes. It is generally accepted that the primary target for radiation effects in biological systems is DNA. However, in addition to the targeted effects of ionizing radiation on DNA, the radiolysis of water and other compounds a human cell result in the formation of ROS which can directly alter DNA through oxidative damage (Dandona et al. 1996). Many RCS are known to damage many biological components in cells other than DNA, including membranes that are essential to the homeostasis of the cell (Bonnekoh et al. 1990; Kryston et al. 2011; Martin et al. 2011). A high concentration of ROS in the cell can critically compromise the mitochondrial membrane and affect the mitochondrial membrane
potential, which is crucial for the production of the majority of aerobic energy in cells (Tada-Oikawa et al. 2000). As reviewed earlier, many studies have linked the production of RCS to mitochondrial dysfunction and to CFIDS, suggesting involvement of factors such as lipid peroxidation, mtDNA damage, pyruvate metabolism, and protein import. Other studies outside of the field have observed very similar events in cells exposed to ionizing radiation, both at high and low doses. Considering the broader metabolic abnormalities in individuals suffering from CFIDS and the link to ATP production (Myhill et al. 2009), there is some compelling evidence in contemporary scientific literature to warrant the association of CFIDS and mitochondrial dysfunction with ionizing radiation exposure and general RCS production. If these connections are juxtaposed with the strong evidence for a biophoton bystander effect as described by Le et al. in Radiation Research (2015) and Le et al. in Physics in Medicine and Biology (2015), it is reasonable to suggest that CFIDS may be aggravated or even caused by low level radiation exposure through a novel signalling cascade.

It is difficult to estimate how many different pathways are linked to mitochondria that might be affected by ionizing radiation either directly or indirectly by bystander signaling. The approach in this paper is to focus on pathways and proteins involved in low dose radiation response that are also suggested as being involved in CFIDS. The particular focus is on involvement in the phenomena of cellular oxidative stress and altered cellular energy metabolism and production.
Biochemical Pathways and Proteins of Interest

Alpha-synuclein

Alpha-synuclein (α-syn) is, by virtue of scientific debate, either an intrinsically unfolded protein (Riedel et al. 2011) or a protein that might undergo folding with the help of heat shock proteins (HSPs) to assume its native conformation in some circumstances (Bruinsma et al. 2011). In humans, α-syn has no known function other than its deleterious role in disease (Riedel et al. 2011). α-syn is known to readily oligomerize, fibrillate, and aggregate, and is the major component of inclusion bodies in cells of patients with Parkinson’s, Multiple System Atrophy, and certain forms of Dementia (Bruinsma et al. 2011). Specifically, the same paper showed that α-syn is known to interact with many HSPs, including αB-crystallin and sHspB8, with different mutations altering the ability of HSPs to prevent fibrilization.

α-syn is thought to have two major forms: a membrane-bound and nonmembrane bound form. This is believed due to the finding that α-syn has the capability to interact with polyunsaturated fats, which, in turn, may help promote the oligomerization process (Riedel et al. 2011). Following this finding, it was postulated that α-syn exists in its nucleating form when bound to membranes and its monomeric form when in the cytosol (Riedel et al. 2011). Because unsaturated fats are known to easily undergo lipid peroxidation after exposure to ionizing radiation (Hammer & Wills 1979), it was also proposed that subsequent α-syn oxidative modification by lipid peroxides and their radical intermediates generated from proximal membrane material could promote its aggregation (Thomas et al. 2007; Riedel et al. 2011).

Studies concerning the tendency for α-syn to aggregate independent of cellular components
after overexpression and ROS introduction respectively have also been conducted (Andrekopoulou et al. 2004; Uversky et al. 2005). Aggregation of α-syn consequently promotes further oxidative stress in the cell (Flower et al. 2005) and could lead to the compromising of broader cellular metabolism. Indeed, increased α-syn levels have already been linked to compromised mitochondrial function and altered cellular metabolism (Hu et al. 2009; Brown et al. 2016a). A recent article (Brown et al. 2016a) demonstrated that α-syn binds to TOM20, a mitochondrial outer membrane translocase receptor, causing deficient mitochondrial respiration, increased ROS production, and loss of membrane potential. Other studies have shown that mitochondrial dysfunction and ER stress can lead to α-syn-mediated cell death (Smith et al. 2005). Another study (Booth et al. 2012) found that blockage of mitochondrial membrane proteins is a potential biomarker of CFIDS. Therefore α-syn should be further examined with regards to its potential role in CFIDS and other diseases.

One study (Saligan et al. 2013) also showed statistically significant correlations between α-syn induction, ionizing radiation, and fatigue symptoms. The extensive study showed that cancer patients exposed to gamma rays by external beam radiotherapy (EBRT) showed elevated α-syn expression after the therapy. The paper also found that the fatigue experienced by patients following the treatment was significantly correlated to α-syn plasma concentration, which indicates that the EBRT produced the same effects on α-syn expression as it did on the fatigue experienced by the patients. While this study alone does not conclusively link α-syn to fatigue and radiation exposure, it raises some important questions concerning its role in cellular metabolism and inflammation in relation to fatigue symptoms. The underlying mechanisms of
this relationship could prove very insightful in the study of CFIDS and its association with ionizing radiation.

In conclusion, α-syn could be a protein that is highly involved in the development or aggravation of CFIDS in many ways. Its link to membranes and lipid peroxidation, mitochondria and cellular metabolism, and radiation treatment-related fatigue symptoms warrant further investigation into its role and relationship to ionizing radiation in the context of CFIDS (Figure 1).

**Lactate Dehydrogenase and STAT1**

Lactate Dehydrogenase (LDH) is an enzyme that is involved in the reaction that converts lactate to pyruvic acid and back using NAD+ and NADH in the cell. Because of its abundance in the cell and because it is mainly released following tissue damage, it is an important marker for measuring alterations to the cell membrane (Bonnekoh et al. 1990; Armeni et al. 2004). Presence of excess lactate has been linked to plasma acidosis and muscle fatigue (Lemire et al. 2008). Studies conducted in the past few years have shown that the importance of LDH may be greater than previously thought, as lactate was identified as a major energy source for human neurons (Hall et al. 2009; Boumezbeur et al. 2011; Wyss et al. 2011). Lactate concentrations are positively correlated with radioresistance, which could indicate intrinsic antioxidant properties (Hirschhaeuser et al. 2011).

LDH subtype A (LDH-A) is an isozyme that is highly expressed in various kinds of malignant tumors and is probably heavily involved in the Warburg Effect (Fantin et al. 2006). The Warburg
Effect is primarily used to describe the metabolism of tumor cells, wherein a switch is made in generating the majority of energy between oxidative phosphorylation and aerobic glycolysis (Vander Heiden et al. 2009). Reduction of oxidative phosphorylation efficiency has been established in CFIDS patients in neutrophils, with a switch to glycolysis postulated after blood samples were taken from CFIDS patients and assayed (Booth et al. 2012). When taking into consideration the fact that mitochondrial oxidative phosphorylation produces ROS, it is thought that this effect limits the production of ROS in normal cells (Vander Heiden et al. 2009). LDH-A is also considered a potential target of cancer suppression. A recent study (Kim et al. 2014) showed that inhibition of LDH-A activity decreases the conversion rate from pyruvate to lactate, and consequently mitochondrial membrane potentials and cellular ATP levels as well; this ultimately leads to oxidative stress in the cell. Reduction of the production of ATP is one of the many biomarkers that have recently been discovered in CFIDS patients’ blood and has been tested extensively (Myhill et al. 2009). A few studies have further linked LDH to mitochondria by indicating that LDH can exist inside mitochondria, contributing, much like cytosolic LDH, to promoting a lactate balance in cells by dehydrogenating lactate produced in the cytosol (Brooks et al. 1999). While there was some debate about the existence of LDH in mitochondria (Rasmussen et al. 2002), a few recent studies have unequivocally confirmed its presence (Lemire et al. 2008; Passarella et al. 2014).

Signal Transducer and Activator of Transcription 1 (STAT1) is a transcription factor in humans, traditionally thought to be a protein involved in interferon signalling. Pitroda et al. (2009) identified STAT1 as a transcription factor of LDH-A, among many other proteins involved in metabolic energy pathways. They suggested that STAT1 works closely to regulate glycolysis and
even demonstrated that STAT1 helps to rescue the expression of proteins essential to metabolic energy pathways after x-ray induced suppression. Another paper studied the exposure of STAT1 to UVA. After exposure to UVA, the modulation of STAT1 expression assumed a biphasic characteristic, according to Mazière et al. (2000). They also found that this effect was reduced with the introduction of antioxidants, indicating the presence of ROS and oxidative stress probably play a role in the modulation of STAT1. Furthermore, the regulation and activation of STAT1 is complicated by the need for tyrosine/serine phosphorylation and the MAPK/ERK pathway, as inhibiting either phosphorylation or the MAPK/ERK pathway inhibits the activation of STAT1. STAT1 is also particularly interesting because of its close involvement with interferon signalling; pertinent to CFIDS, STAT1 is involved downstream of interferon gamma, a signalling molecule modulated and typically decreased in lymphocytes of patients with CFIDS, in one pathway (Klimas et al. 1990; Visser et al. 1998; Patarca 2001; Mulero et al. 2015; Baris et al. 2016). The potential role of STAT1 in regulating cellular metabolism and LDH-A could suggest its involvement in a variety of diseases, in addition to its link to interferon signalling in the context of CFIDS. Moreover, the ability of ionizing radiation to affect, let alone modulate, the expression of STAT1 is cause enough for further research into the effects of ionizing radiation on LDH and its regulators, the interplay of ROS, and ultimately the pathways’ possible contribution to CFIDS. Further discussion of STAT1 in relation to the expression of RNase L is discussed in the Ribonuclease L section.

Studies have also shown that radiation-induced senescence of human breast cancer cells also caused the upregulation and activation of LDH (Liao et al. 2014). The study by Liao and his team also showed that, by altering the monocarboxylate transporter 1 (MCT1), adenosine
monophosphate-activated protein kinase (AMPK), and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathways, radiation can effectively induce the senescence of cells by metabolic alterations. The AMPK pathway is particularly interesting, as it mediates the cellular response when the AMP/ATP ratio becomes too high as the cell experiences energetic stress. The study also showed that invasion of surrounding cells and radiation-induced bystander signalling was dependent on the MCT1, AMPK, and NF-κB pathways. Recent studies have linked the NF-κB pathway and loss of p53 directly to CFIDS (Morris & Maes 2012). A very interesting review proposed a common etiology between post-radiation syndrome and CFIDS. Pall (2008) described post-radiation syndrome as a CFIDS-like disease and linked the entire illness to the nitric-oxide/peroxynitrite cycle (NO/ONOO–). The it was proposed that the cycle involves NF-κB and interferon gamma signalling and oxidative stress, culminating in a “vicious cycle” that chronically elevates levels of nitric oxide. It was also concluded that radiation can, even at relatively low doses such as those experienced by Chernobyl recovery workers, can lead to the activation of this cycle, primarily through NF-κB.

Moreover, another study (Badalà et al. 2008) found elevated ventricular lactate in CFIDS patients and implicated oxidative stress as a cause of the disease. These findings suggest that, while CFIDS may be a complicated disorder with many different symptoms, more research must be done in order to rule out the direct involvement of LDH and its associated proteins in the illness.

LDH is an important protein with respect to cellular metabolism and energy production. The involvement of LDH and its associated pathways in the Warburg Effect, oxidative stress, mitochondrial structural and functional integrity, STAT1 regulation pathways, and radiation-
induced activation and senescence all necessitate future endeavours to fully understand its potential role in contributing to CFIDS symptoms (Figure 2).

**Cytochrome c and Cytochrome c oxidase**

Cytochrome c oxidase (CCO) is a large multi-protein complex located in the mitochondria of eukaryotes. Also known as Complex IV, it is a main component of the electron transport chain, where it donates four electrons to molecular oxygen and transfers four protons to it as well, creating two molecules of water in the process. In addition to this, it pumps protons into the intermembrane space of mitochondria to create the electrochemical gradient required to make ATP (Denis 1986). Cytochrome c (CC) is a hemeprotein associated with the inner membrane of mitochondria; essential for the electron transport chain as well, it has the capacity to carry a single electron, to donate that electron during oxidative phosphorylation, and to shuttle electrons between Complex III and Complex IV.

CC is crucial in many pathways in the cell. Its release from the mitochondria following mitochondrial permeabilization is necessary for many pathways leading to apoptosis (Yang 1997). Its involvement in the electron transport chain makes its uncompromised role an important factor in the viability of cells with respect to energy production. CC has the capability to produce intramitochondrial ROS when interacting with several proteins, leading many to believe that it is involved in signalling and mitochondrial damage through ROS-propagation pathways (Giorgio et al. 2005; Marchi et al. 2012; Panieri & Santoro 2016).
A study by Tirelli et al. (1994) indicated that there were no significant differences in CCO reduction between control groups and CFIDS patients. Indeed, a few other older scientific articles note no significant disparities between the amount of CCO reduction in healthy individuals versus CFIDS patients, particularly in muscle tissue where the energy demand is high for cells (Byrne & Trounce 1987). However, more recent publications have noticed some important connections between CCO and CFIDS.

The aforementioned link that RCS have to mitochondrial dysfunction and CFIDS has been studied extensively in the literature. A study (Brown 2001) found that CCO can be reversibly impaired by a radical nitrogen species, NO in this case, as CCO competes with it for access to oxygen. It noted that prolonged or high dosages of NO or any of its derivatives can cause irreversible deleterious effects, such as CCO’s uncoupling, mitochondrial permeability, and even cell death. Another article noted that CC can be modified by nitration of its tyrosine residues after low doses of peroxynitrite are administered (Marchi et al. 2012). The authors found that the nitration of CC also resulted in the upregulation of peroxidase activity for hydrogen peroxide in the cell and the impairment of membrane potential formation, likely a result of damaged subunits in the electron transport chain. Oxidative damage to mitochondrial proteins and complexes crucial to oxidative phosphorylation not only results in impaired ATP production, but also unregulated ROS production by the affected complexes, which leads to amplification of ROS in mitochondria and increased release into the cell (Hosseini et al. 2014; Zorov et al. 2014). It has been shown that the production of peroxynitrite and its derivatives, NO included, can be induced by x-ray exposure in mice (Hanaue et al. 2007), which could potentially lead to CC nitration, multi-complex corruption, RCS proliferation, mitochondrial
membrane depolarization, and reduced ATP production leading to ROS contribution to fatigue and even tissue insult as a result of mass cell death. The theme that the nitric oxide cycle and elevation of NO has something to do with CFIDS in humans is pervasive in the literature; many papers have been published on the topic, including a few linking the nitric oxide cycle to radiation exposure and PRS (Pall and Satterlee 2001; Pall 2007, 2008, 2009).

The biggest problem with studying CC and CCO is their nature as incredibly complicated macromolecules, particularly CCO. Be that as it may, the roles that they might have in CFIDS should not be underestimated. Both molecules are already being studied heavily, and the fact that have already been implicated in a number of diseases only further necessitates research. The discovery of RCS signalling pathways and the involvement of CC in them, the potential for exogenous RNS to affect the metabolism of mitochondria through direct covalent interactions with CC and competition with CCO, and the demonstrated radiation-induced production of a reactive species of nitrogen implicated in these cascades in vivo (Figure 3) beckons the need for further research into CC and CCO.

**αB-crystallin**

αB-crystallin (αBC), encoded by the gene CRYAB, is a protein that is a member of the small heat shock protein (sHsp) family and is involved in binding to unfolded proteins to inhibit their aggregation and ultimately preventing apoptosis (Boelens 2014). αBC interacts with many proteins, inhibiting the formation of mature α-syn fibrils (Hsu et al. 2000; Riedel et al. 2009), reducing the ubiquitination and degradation of a pore-forming subunit of major cardiac voltage
gated sodium channel (Huang et al. 2016), and binding to p53 in addition to several pro-apoptotic proteins and preventing their translocation to mitochondria (Liu S et al. 2007). In fact, the interplay between p53 and αBC shows tight regulation on a transcriptional and post-translational level and affects oxidative stress and the Warburg Effect in human cells. It was discovered that p53 binds directly to the promoter region of CRYAB to enhance its expression; transfection of a p53 overexpression vector into MCF-7 cells increased expression of αBC, reduced intracellular ROS, caused the Warburg Effect, and increased ROS after p53 was depleted (Liu S et al. 2014). It was also shown, as stated previously in the LDH section, that reduced levels of p53 is indicative of mitochondrial dysfunction and directly linked to CFIDS. Many sHSPs are mobilized during oxidative stress (Dimauro et al. 2016), and in case of αBC, a few crucial mitochondrial pathways involved in ROS production and cell damage might be one of the major reasons for this.

αBC was shown to be modified heavily by the reactive nitrogen species peroxynitrite, where peroxynitrite enhanced its chaperone and antioxidant abilities (Morris & Maes 2012). This is very interesting when noting that peroxynitrite is heavily involved in compromising CC and CCO, as discussed in the Cytochrome c section, and is thought to be central to the etiology of CFIDS and PRS (Pall and Satterlee 2001; Pall 2007; Meeus et al. 2008; Pall 2008, 2009). Specifically, there appears to be the potential for rescue in the Cytochrome c and Cytochrome c oxidase-mediated ROS pathway after radiation exposure when considering the scientifically-corroborated observation of radiation-induced formation of peroxynitrite (Zorov et al. 2014).

αBC was also shown to inhibit the release of Cytochrome c from mitochondria (Xu et al. 2013), potentially by binding to mitochondrial transmembrane channels. As shown by Chis et al.
Cytochrome c co-localizes with αBC during periods of oxidative stress, as are other pro-apoptotic proteins. αBC was also shown to have antioxidant properties by binding directly to Cu2+ ions and rendering them inactive (Ahmad et al. 2008). Crystallin genes have been shown to be highly induced following radiation exposure in human cells and are closely linked to mitochondria-stress pathways (Andley et al. 2000; Parcellier et al. 2005; Chaudhry 2006).

Could αBC be implicated in CFIDS? While the connections made to CFIDS and radiation may not be conclusive, this chaperone is clearly involved with many aspects of proper cellular function. The fact that αBC is involved in regulating every protein discussed in this paper, the fact that increased αBC is correlated with increased lactate levels and the Warburg Effect, and the fact that is relatively sensitive to ionizing radiation only speaks to the need to research αBC with respect to radiation and CFIDS (Figure 4).

Ribonuclease L

Ribonuclease L (RNase L) is a ribonuclease that, upon activation, destroys RNA that resembles viral RNA. RNase L is mobilized after either a type I interferon signalling cascade involving the stimulation of JAK kinases and after activation of STAT1 and/or STAT2 results in transcription of 2,5A Synthase (2-5OAS); this synthase is activated by ss and dsRNA to create 2′-5′-oligodendylates (2-5A), which bind to and activate RNase L (Stark et al. 1998; Silverman 2007a). RNase L is also believed to be involved in apoptosis, specifically during the cell’s efforts to enter apoptosis following viral infection (Chakrabarti et al. 2011). Indeed, after a certain point of
activation, RNase L is capable of destroying essential RNA in the cell in an attempt to induce apoptosis to prevent the infection from spreading (Silverman 2007b).

It has been believed for some time that RNase L cleavage is linked to CFIDS. The protein exists in its functional 83 kDa form in both CFIDS and healthy groups. Various truncated forms of RNase L also exist, albeit mostly in unremarkably similar concentrations in both groups. Curiously, some studies suggest that the 37 kDa form of the protein exists preferentially in CFIDS groups (Suhadolnik et al. 1997; De Meirleir et al. 2000). One study in particular attempted to link the upregulated activity of elastase to the cleavage of RNase L (Meeus et al. 2008). The same study found significant amounts of truncated 37 kDa RNase L in patients with CFIDS. Upregulation of 2-5OAS has been directly linked to CFIDS as well, and one study found a significant correlation in the expression of 2-5OAS with fatigue and depression symptoms stimulated by interferon alpha treatment in patients with Hepatitis C (Felger et al. 2012).

RNase L has been shown to have capabilities to cleave 28s rRNA in mice as part of an attempt to prevent viral proliferation (Iordanov et al. 2000); another study showed RNase L-independent 28s cleavage can occur during viral infection (Banerjee et al. 2000). A study by Iordanov et al. (2000) suggested that the degradation of 28s rRNA, which makes up the large ribosomal subunit, could lead to overall reduced translation in the cell and induction of pro-inflammatory cytokines to the cell as part of the innate immune response. Such an implication would mean that the loss or dysregulation of RNase L in any way could lead to an inappropriate response to viral infection and serious immune dysfunction, which could be a route to developing the immune symptoms present in CFIDS.
The possibilities for the involvement of radiation in this pathway are virtually limitless and mostly unexplored in the literature. For instance, it has been shown that RNase L plays an important role in upregulating the cellular response to gamma radiation-induced DNA damage by affecting the transcription of proteins such as p21 (Al-Haj et al. 2012). It has been shown through several studies that various kinds of kinases, including MAP and ATM kinases, can be activated by ionizing radiation to phosphorylate their targets (Chen et al. 1996; Canman et al. 1998; Iordanov et al. 2000). Results from the Maziere et al. paper (2000) imply that the biphasic modulation of STAT1 following radiation exposure might very well have something to do with MAPK, JAK, or other similar tyrosine kinases, as inhibition of tyrosine kinase activity inhibited the UVA-effect on STAT1. STAT1 is required for the transcription of 2-5OAS, which is essential for the activation of RNase L among other interferon response genes in the Type I pathway expressed throughout the human body in a multitude of tissues during viral infection (Mullan et al. 2005). STAT1 is also necessary and sufficient to induce transcription of interferon-stimulated genes in the Type II pathway specific to Type II interferons released from activated T lymphocytes (Durbin et al. 2000; Mullan et al. 2005).

As described in the STAT1 section, it is important to note that there are many potential pathways that may link radiation to its pathway and eventually CFIDS. Dysregulation of the RNase L pathway is an interesting possibility for the manifestation of CFIDS. Many questions still remain unanswered in this pathway with regards to radiation and CFIDS, necessitating further research.
Potential New Areas for Research

The literature discussed above confirms that ionizing radiation has been shown to be capable of perturbing various pathways thought to be involved in the manifestation of CFIDS. Some areas for future research are discussed below in the hope of contributing to our understanding of CFIDS and its potential connection to radiation exposure.

Involvement of the Bystander Effect

Because of the potential for signal propagation through cell populations (Nagasawa and Little 1992; Mothersill and Seymour 1998, reviewed in Mothersill et al. 2017a; 2017b) as a mechanism which can magnify the size of the target for effects after low doses, the following experiments might help to determine if the effects can be extrapolated to living multicellular organisms following localized radiation exposure. Such studies could lead to a better understanding of the potential for cellular symptoms of radiation to proliferate in a cell population to affect the system as a whole. Comparison of results with and without the compounds discussed above would enable us to determine if the radiation response pathway were actually impacted by the interference with the biochemical pathway.

The Peroxynitrite Cycle

The effect of the presence of peroxynitrite on αBC, CC, and CCO (Brown 2001; Andrekopoulos et al. 2004) could be a primary focus as a potential pathway in the proliferation of ROS, mitochondrial and cell damage, energy deficiency, and apoptosis. As noted earlier, the
formation of peroxynitrite can be induced by x-ray exposure, particularly in mice (Hanaue et al. 2007), and a few papers (Pall 2007, 2008, 2009) have noted that RNS pathways could be the missing link between CFIDS and radiation exposure in humans.

The first step would be to identify a human cell line where the production of peroxynitrite occurred. This would be of high interest with regard to CFIDS. A control with established baseline concentrations of peroxynitrite and its derivatives would have to be determined, before looking at the impact of ionizing radiation on other samples. The baseline production of other ROS and RNS species could be assayed as well in this step. If initial results are promising an experiment attempting to correlate the radiation dose, the peroxynitrite concentration, and the nitration of Cytochrome c could be performed. Assaying the modification of Cytochrome c could be done using sodium dodecyl sulfate polyacrylamide gel electrophoresis using a molecular weight ladder and comparing the untreated lane to the varying radiation dosages/concentrations of peroxynitrite. Using SDS-PAGE in this way could show a shift in mass of cytochrome c between the untreated samples and the treated samples, answering the question of whether increased radiation exposure leads to increased nitration of Cytochrome c. If this does not work due to the change in mass being potentially too small to detect, other methods could be discussed. Again, this experiment would only attempt to establish whether increasing doses of gamma radiation exposure alter Cytochrome c through nitration mechanisms.

Using immortalized human cell lines, preliminary clonogenic assays could potentially show the reduction in viability of cells that undergo this kind of alteration to Cytochrome c, which could potentially link the alteration to damaged electron transport chain (ETC) components.
Bystander signals are already known to impact the ETC (Le et al. 2017) This step would putatively link reduced viability caused by radiation exposure to cellular respiration based on Cytochrome c’s intimate involvement in oxidative phosphorylation. A direct assay of the alterations in each Complex by various methods might reveal some damage as well, which would support the theory, although not directly. An assay of the functionality of Cytochrome c in irradiated cells would have to be done to implicate it in reduced cell viability.

αB-crystallin could be involved in several ways in this process. It is suspected, based on literature review, that alterations to αBC by peroxynitrite enhance its antioxidant and chaperone properties. There is the potential to measure the rescue of Cytochrome c by showing that αBC binds directly to Cytochrome c after irradiation and consequent nitration, as it has been shown to co-localize with unaltered Cytochrome c (Chis et al. 2012). This could be confirmed using a co-immunoprecipitation assay. This would involve isolating modified Cytochrome c using an antibody, then identifying potential binding partners using a Western Blot in the hopes of identifying αBC. It would also be interesting, assuming Cytochrome c is bound by αBC after irradiation, if the affinity increases or decreases with more nitration and/or exposure to radiation.

Because Cytochrome c oxidase is involved in competition with nitrogen species, this could be a novel mechanism induced by ionizing radiation that could be linked to oxidative stress and CFIDS in the future. Considering that ROS production, Cytochrome c, and αB-crystallin are involved in cell metabolism, energy production, and have been linked to CFIDS in some way, this pathway that involves the production of ROS, the loss of function of Cytochrome c, and αB-crystallin is interesting when considering its ties to ionizing radiation. This experiment would
clearly not determine if this pathway operates in vivo in those suffering from CFIDS, however elucidation of these pathways and their associated proteins is most definitely a good start.

P53, αB-crystallin, and ROS

Some very interesting regulation can be found in the p53 and αBC pathway. It appears as though αBC negatively regulates the apoptotic functionality of p53 (Liu S et al. 2007). P53 positively regulates the expression of αBC (Liu S et al. 2007; 2014) and a number of proteins that probably interact with other proteins involved in cell metabolism in order to induce the Warburg Effect (Morris & Maes 2012; Liu S et al. 2014; Panieri & Santoro 2016).

No published experiments have yet assayed αBC’s resilience to gamma radiation using small doses. It would be interesting to irradiate a solution containing αBC in the soluble fraction at low doses, run it on an SDS-PAGE gel, determine if there are any appreciable changes, and then determine what percentage of αBC was lost due to the radiation compared to the control. The same experiment could then be done, with cells. The cells could then be lysed, αBC isolated, and then measured. This would be done alongside unirradiated controls to determine if the fraction of functional αBC was appreciably altered. Clonogenic assays would be done at each endpoint to determine if this effect can be correlated with survivability. If the results between the experiments using the chemical alone or the chemical in cells are different, then we can postulate that ROS had a role in the damage done to αBC in the cell. It could suggest that ROS generated inside cells might have played a role in αBC’s reduction. This would suggest that certain photosensitizers might help in the propagation of ROS and the damage that they do
inside cells. If the converse was seen, then some compound or protein in living cells might be protecting αBC from alterations caused by radiation that is not present in the chemical solution. Further studies could be done to determine what radioprotector this might be, potentially by reviewing the literature to identify putative binding partners and using coimmunoprecipitation to validate them.

Considering that there are p53 +/- and p53 -/- cell lines available, similar experiments can be done on both cell lines. Firstly, the disparity between cells with no p53 and cells with p53 concerning expression of αBC would have to be established. Then, experiments could be done in a similar fashion as above to try to link the reduction of αBC to pro-apoptotic pathways involving p53, a shift to more glucose in the cell, and ROS production. Even though these experiments would be performed on human cell lines and the results would not be directly applicable to CFIDS patients, determining how these pathways behave in certain situations could give researchers more potential biomarkers to observe in CFIDS patients after follow-up studies testing for relevance in such patients.

**Lactate Dehydrogenase and the Warburg Effect**

In one reviewed study, it was shown that ventricular lactate levels were significantly higher in people with CFIDS (Badalà et al. 2008). This raises the question of whether or not rising intracellular and intercellular lactate levels have a role to play in CFIDS. Perhaps damaged mitochondria due to ROS induce a cellular response to use more aerobic glycolysis in the cytosol while some mitochondria shut down. The problem with LDH is that it can be involved in
a number of ways in the onset or aggravation of CFIDS: LDH could protect the cell from oxidative damage, facilitate the switch to aerobic glycolysis if the mitochondria are damaged, or facilitate the switch indirectly through a number of different pathways.

The mechanisms of such a switch could be triggered by radiation exposure. It has been shown that lactate could be a radioprotective molecule (Hirschhaeuser et al. 2011) and that LDH-A can induce oxidative stress if it is downregulated in cancer cells (Panieri & Santoro 2016). If LDH is, in fact, an indirect radioprotective element in normal cells, then its loss would render cells more susceptible to radiation. To determine if ROS play a major role in the radioprotective nature of LDH, an assay measuring the antioxidant properties of LDH might be appropriate where radical chemical species’ levels are being measured. A simple experiment would be to perform an assay in a solution where RCS can be generated by radiation. Different doses of radiation can be applied to each flask. After irradiation and measuring of relative reactive species in the solution, LDH can be added to each with varying concentrations. The assay would be performed again to measure RCS. If the trials show reduced RCS, then it could suggested that LDH is sufficient as an antioxidant and can protect against radiation-induced generation of RCS irrespective of the presence of other proteins or chemicals. If no significant reduction is seen, then LDH might not be an antioxidant at all; much more likely than this however, is that LDH does not have intrinsic chemical antioxidant properties, but might reduce reactive species in the cell in the proper chemical environment and when assisted with auxiliary proteins. The same set of experiments can be done again, but this time with LDH present in the solution before irradiation. If a significant difference in ROS is seen between the two groups, then LDH might be adversely or positively affected by radiation directly, which contributes to its
functionality in a cellular environment. If there is no significant difference between the two groups, then it can be concluded that LDH is not directly affected by ionizing radiation at the doses and wavelengths used.

Another set of experiments could follow testing the effects of ionizing radiation of LDH in living cells. These sets of experiments would involve irradiating cells and will attempt to link ionizing radiation and generation of RCS to radioprotective effects in vivo. This would involve irradiating cells at the same dosages as in the first set of experiments mentioned above. Assays measuring the loss of LDH and generation of RCS would then be used to attempt to correlate the two. If the results are positive, then the loss of LDH should correspond to the increase of RCS in the cell. This would imply that the loss of LDH as a radioprotector might lead to the increase of RCS in cells. In order to confirm this, a knockdown of LDH by means independent of radiation would have to be performed to directly implicate the loss of LDH to increased RCS caused by radiation in vivo. There are a few methods that could accomplish this, but the most direct one would involve the use of RNAi. If this method is chosen, then LDH would be knocked down in cells by RNAi and then subjected to radiation. The levels of LDH and RCS in the dosage groups would have to be measured. When this experiment is complete, one could compare the numbers to the previous experiment where RNAi-induced knockdown of LDH was not performed. If significant disparities are seen between the groups, then it can be better concluded that LDH plays a direct role in reducing RCS in human cells, either intrinsically or through some pathway involving other proteins or chemicals.
To take these experiments further, clonogenic assays should also be done to correlate this effect to survivability. Lactate levels can also be measured in cells which would be anticipated to go up following knockdown of LDH. The effects of this on cellular metabolism can also be measured; membrane potentials of mitochondria can be measured using the JC-1 dye or another stain for membrane polarization, the functionality of different complexes can be assayed before and after, and so on. Every subsequent experiment would attempt to link the reduction of LDH and radiation to metabolic dysfunction and contribute to identifying the potential causes of cellular markers of CFIDS.

**Alpha-synuclein and ROS**

Researchers believe that the aggregation of α-syn causes ROS formation (Hsu et al. 2000; Uversky et al. 2005; Boelens 2014). In a similar fashion, ROS production causes α-syn aggregation (Hsu et al. 2000; Uversky et al. 2005; Riedel et al. 2011). One article suggests that the proximity of α-syn to lipid membranes contributes to its oxidative modification upon lipid peroxidation after irradiation, causing it to aggregate (Riedel et al. 2011). A few tests could correlate the two. The purpose of these experiments would be to determine if α-syn can undergo aggregation after irradiation, what mechanisms underlie this aggregation process, and what effects are produced on the cell.

The first step would be to measure lipid peroxidation by some kind of assay after irradiation in cells. There are several assays available to measure lipid peroxidation, with one of them being an LDH assay. This would be done in live cells. Next, one could extract α-syn and run it on a gel
to determine how much was lost when compared to unirradiated controls. Next, one could irradiate extracted α-syn in solution at different doses and run that on a PAGE gel to determine if any significant alterations occurred. After this experiment is complete, an experiment should be performed where membrane tissue is isolated from human cells. One could then incubate extracted α-syn with membrane tissue, irradiate at different dosages, purify the α-syn solution, run it on an SDS-PAGE gel, and determine if there are any differences between this gel and the one with α-syn alone. This would tell researchers if presence of fatty tissue affects the alteration of α-syn by radiation. This would directly link lipid peroxidation to α-syn alterations if the results are positive. If the results are negative, the finding that radiation-induced lipid peroxidation, radical propagation, and termination in α-syn is negligible, and alterations happen either directly or through the presence of another protein or species that can oxidize α-syn.

After this experiment, another experiment would consider the radiation-induced alterations of α-syn in vivo using similar methods as above. There are a few possibilities in the outcomes of each experimental sequence. The following lists all of the possible results and conclusions following the full sequence of experiments:

1. Incubating and irradiating α-syn together with lipids produces no significant change in the alterations of α-syn

   **Conclusion:** Lipid peroxidation mechanisms are negligible in radioactive alterations to α-syn.

   a. Irradiating live cells containing α-syn produced no significant change in the alterations of α-syn compared to *ex vivo* trials
Conclusion: If different than unirradiated controls, then α-syn probably undergoes alterations caused by radiation directly. If the same as unirradiated controls, then radiation probably has no significant effect on the alteration of α-syn, both in vivo and ex vivo.

b. Irradiating live cells containing α-syn produced a significant change in the alterations of α-syn compared to ex vivo trials

Conclusion: While lipid peroxidation mechanisms are negligible, some other mechanism exists that affects the oxidation of α-syn. This probably occurs by photosensitizer species and/or other proteins; further investigation is needed to identify these.

2. Incubating and irradiating α-syn together with lipids produces a significant change in the alterations of α-syn

Conclusion: Lipid peroxidation mechanisms are at play in radioactive alterations to α-syn.

a. Irradiating live cells containing α-syn produced no significant change in the alterations of α-syn compared to ex vivo trials

Conclusion: Lipid peroxidation is the primary pathway that is needed for oxidative alterations of α-syn.

b. Irradiating live cells containing α-syn produced a significant change in the alterations of α-syn compared to ex vivo trials
Conclusion: Lipid peroxidation is one of the primary pathways that are needed for oxidative alterations of α-syn. Other mechanisms affect the oxidation of α-syn; further research is needed to identify these pathways.

It is important to note that the above results would not be mutually exclusive, as a dosage threshold may have to be reached before any one of these deterministic effects are observed. It is also important to note that, due to the findings by Saligan et al. (2013), radiation dosages can cause elevated α-syn concentrations; this will need to be accounted for by using many controls and measuring quantity of protein in the PAGE gels by comparing them to the concentration of the molecular ladder. If this method is deemed too inaccurate, another assay measuring protein expression must be done for the controls before the experiment and for the test groups after the experiments.

Clonogenic assays would be done at each endpoint in the in vivo trials to determine if survivability was affected. These assays would help correlate the survivability of cells with reduced α-syn functionality in the trials. Further experimentation can be done to elucidate the other mechanisms that might affect the radiation-induced dysfunction and aggregation of α-syn where applicable, and implicate the effects of bystander signalling and radioprotectors in these results in future studies.

Overall, the findings in these experiments would contribute to understanding one pathway in which CFIDS could potentially manifest. The experiments described above would be relying on the ideas described in Saligan et al. (2013), wherein the group described elevated fatigue symptoms with radiation therapy and increased expression of α-syn. Considering that
subsequent studies will be needed to determine if this effect is relevant to CFIDS patients and even if α-syn radiation-induced transcriptional and post-translational alterations cannot be linked to fatigue symptoms for quite some time, these experiments will be a crucial first step in determining the effects of radiation on α-syn from a molecular perspective.

A multitude of other experiments must be pursued in order to further understand the involvement of these pathways in CFIDS, as these experiments are merely suggested directions for future research. No one study will conclusively validate or disprove the connections that these proteins have to CFIDS. Again, the purpose of all of these proposed experiments would not be to indisputably and unassailably link any of the mentioned proteins to CFIDS, but rather to help elucidate these pathways further and potentially give other researchers background studies to begin making connections that could not be made before, potentially to CFIDS and the influence of radiation.

**Conclusion**

Alpha-synuclein, cytochrome c, cytochrome c oxidase, lactate dehydrogenase, STAT1, and αB-crystallin are but a few proteins potentially involved in the development of Chronic Fatigue Syndrome. When taking into consideration the very real and exciting possibility of signal propagation in cell systems via bystander signalling, the effects of radiation on the disease might involve a completely novel mechanism of radiation-induced pathogenesis that could have drastic implications in not only Chronic Fatigue Syndrome, but a number of other diseases as well. Numerous very interesting biochemical pathways have been identified through literature
review that could shed further light on the mechanisms behind Chronic Fatigue Syndrome in the context of ionizing radiation, radical chemical species formation, cellular damage, and apoptosis.

The beauty of modern biology is that it allows scientists to investigate innumerable biochemical pathways involved in diseases that will inevitably converge on a set of causes for the disorder. While the proteins under investigation have a broad range of potential functions in the cell, determining the connection between these proteins and the metabolic and cellular characteristics of Chronic Fatigue Syndrome may provide invaluable insights into the disease. Understanding the molecular biology and biochemistry behind a particular disorder paves the road to patient studies, the development of targeted treatments specific to one or more pathways, therapy options, clinical trials, and eventually a treatment. In this circumstance, taking a broader approach by targeting processes and proteins shown to be involved in Chronic Fatigue Syndrome as well as many cellular characteristics of disease will identify targets for more research, and will, eventually, lead to a substantiated and comprehensive model for a disease that has burdened too many for far too long.

Acknowledgments

The CFIDS Foundation Inc. who funded this review and in particular the director - Dr. Alan Cocchetto who pointed the authors in the direction of many of the papers covered by the review and provided encouragement and ideas.

Declaration of Interest

The authors declare no conflicts of interest.
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