Review Article

Review on cytochrome C

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Abstract

Cytochrome c (Cyt c) is an essential mitochondrial protein which takes part in the electron transport system and apoptosis. Cyt c also scavenges reactive oxygen species (ROS) and oxidizes cardiolipin during apoptosis. The recent finding that cyt c is phosphorylated *in vivo* for the important role of cyt c in the regulation of making life and death decisions. An apoptotic sequence of events takes place which involves changes in cyt c phosphorylation, increased ROS through increased mitochondrial membrane potentials, the oxidation of cardiolipin by cyt c, and its release from the mitochondria. Cyt c regulation in respiration and cellular death is discussed about human disease which includes neurodegenerative and cardiovascular diseases, cancer, and sepsis.

Keywords: Apoptosis, cancer, cardiolipin, cytochrome c, oxidative phosphorylation, reactive oxygen species

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INTRODUCTION

Mitochondria also called as the "powerhouse" of the cell. It is the main site for the synthesis of adenosine tri phosphate (ATP). It also plays a very important role in oxidative phosphorylation. Two proteins which are found in mitochondria are cytochrome c (cyt c)[1] and apoptosis-inducing factor (AIF)[2] which are the main signaling molecules of apoptosis.[3] Mitochondria help in energy metabolism,[4] ion balance,[5] and help in the maintenance of redox potential.[6] Cyt c is a globular protein which contains iron porphyrin cofactor (heme c) which is linked with the polypeptide chain by covalent bond. Cyt c takes part in the electron transport chain (ETC) which happens in the inner mitochondrial membrane. It plays a crucial role in cellular respiration. In the ETC, when an electron is transferred from ubiquinol-cyt c reductase (complex III) to cyt c oxidase (complex IV), cyt c undergoes a reversible reaction, simultaneously gets reduced and oxidized too. The site of interaction between cyt c and complexes III and IV consists of the central hydrophobic area and the surrounding electrostatic area.[6,7] cyt c contains 104 amino acids in mammals. It is a positively charged protein. It is a multifunctional enzyme which takes part in the life and death decisions of the cell. It is essential for the formation of the apoptosome and its progression to apoptosis. Other than these two functions, the other functions of cyt c include its function as a cardiolipin peroxidase,[8] and the detection of four phosphorylation sites on Cyt c, reveals its multifunction are regulated by cell signaling pathways.[9]

STRUCTURE OF CYTOCHROME C

Cyt c which is a protein present in the inner membrane of the mitochondria was one of the first proteins which were seen in X-ray crystallography.[10] The heme group is covalently bonded to the peptide chain by thioether bonds with cysteine

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residues 14 and 17.[11] The iron which is present in the heme is present in a hexacoordinate structure with His18 and Met80 as amino acid ligands.[12] The bonding between iron of the heme and Met80 is responsible for weak 695 nm absorption band in the spectrum of cyt c in the oxidized state. Many aliphatic and aromatic amino acids have been identified in the side chains which make the heme group in a hydrophobic environment, which together with the iron ligands His18 and Met80 are responsible for the high-redox potential of cyt c.[12-14]

FUNCTION OF CYTOCHROME C IN OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation is the last step in cellular respiration where ATP is generated. This process takes part in the inner mitochondrial membrane and consists of ETC and ATP synthase.[15] Several substrates take part in oxidative phosphorylation, including nicotinamide adenine dinucleotide(Reduced) NADH and flavin adenine dinucleotide (Reduced) FADH2. While the transfer of the electrons, the ETC generates the mitochondrial membrane potential ($\Delta\Psi m$). ETC complexes I (NADH dehydrogenase), III (bc1 complex), and IV (cyt c oxidase, CcO) pump protons from the mitochondrial matrix into space between the inner and outer mitochondrial membrane (OMM).[1,16] This mitochondrial membrane potential is utilized by ATP synthase (complex V) in the final step, for the production of ATP.[17] ATP synthase converts the membrane potential into rotational and chemical energy by joining a phosphate and an ADP molecule into ATP.[18] Cyt c is located in the intermembrane space and functions as a single-electron carrier from the bc1 complex to CcO in the final step of the ETC.[19] In this step, reduced cyt c transfers an electron to CcO and it goes on for four times, after which one oxygen molecule is reduced to water. In mammalian cell, this reaction is the rate-limiting step of the ETC under physiological conditions.[20] The electron transfer that takes place from cyt c to oxygen through CcO, converts oxygen to water with free energy of $\Delta Go' = -100$ kJ/mol, which is about twice as high as compared to the reactions catalyzed by complexes I and III.[21] Since this is an irreversible reaction, this step of the ETC should be tightly regulated. This hypothesis is supported by the fact that all major regulatory mechanisms are present, including allosteric regulation of CcO and cyt c the expression of tissue-specific isoforms of cyt c and six subunits of CcO, and reversible posttranslational modifications such as phosphorylations, which have been identified in all mammalian OxPhos.[22]

ROLE OF CYTOCHROME C IN APOPTOSIS

The importance of cyt c during development and life is not only in the function of ATP production and as a radical scavenger but also its essential role in apoptosis. The first report showing that cyt c plays an important role in the cell death pathway was published in 1996 using a cell-free apoptotic system to which compounds were added, such as cyt c and deoxy adenosine triphosphate (dATP), another factor required apoptosis.[23] Other work suggested that molecular changes at the level of cyt c, but not its degradation, occurred during apoptosis.[24] Since many studies have been published confirming those initial findings. It is now accepted that a key step in the apoptotic cascade involves the release of cyt c into the cytoplasm where it binds with apoptotic protease-activating factor 1. Binding of cyt c results in an increased affinity of the complex for dATP whose binding is necessary for oligomerization and formation of the apoptosome.[25] The apoptosome, in turn, recruits many procaspase-9 molecules and promotes their cleavage to an active form, known as the initiators of apoptosis. Caspase-9 bound to the apoptosome acts as the cleavage factor of caspase-3, which is otherwise considered the major enzyme in the committal to apoptosis.[26] Spectroscopic methods allow the real-time measurements of cyt c distribution in the cell based on the changes in its redox state, when it is released into the reducing cytosolic environment during apoptosis.[27] Cell lines without cyt c exhibit reduced caspase-3 activation when stimulated with apoptosis-inducing agents,[28] which show that cyt c is essential for caspase activation. Therefore, cells without cyt c will not only have decreased metabolic rates but also will be nonresponsive to stress signals that would induce cell death. Other studies have suggested that the release of cyt c initiates apoptosis by binding inositol trisphosphate (IP3) receptors, causing calcium release into the cytosol[29] and subsequent calpain activation and AIF release,[30] it has long been known that isolated mitochondria can reversibly release and take up cyt c, while the latter restores their function.[31] Cyt c can be selectively released from the mitochondrial intermembrane space in a manner that does not involve membrane rupture or mitochondrial permeability transition pore opening.[32,33] The release of cyt c takes place before permeability transition[34] and is accompanied by a sharp increase in reactive oxygen species (ROS) production that involves complex I,[35] thus elevated ROS could serve as an initiator for permeability transition.

An important example for the reversibility of cyt c release to restore ETC activity was demonstrated in an animal model for sepsis. Sepsis is a systemic inflammatory condition and

it can lead to multiple organ failure and even death. In an animal model for sepsis, the authors found inhibition of oxidative phosphorylation at the level of cyt c and CcO.[36] The intravenous injection of cyt c led to an uptake of cyt c and significantly improved mitochondrial function, and survival increased from 15% for the controls to about 50% in the septic animal that received the cyt c injections. At first, these findings seemed little puzzling, because cyt c not only has to be taken up by the mitochondria but it also has to go across the cell membrane of the cell. It was recently shown that cyt c contains cell-penetrating peptide (CPP) epitopes in the N- and C-terminal helices.[37] Such CPPs help in the cellular uptake of proteins and may explain that intravenous injection of cyt c can restore ETC function.

FUNCTION OF BCL-2 PROTEINS AND CYTOCHROME C IN APOPTOSIS

The first step for the initiation of apoptosis in mitochondria is mediated through Bcl-2 family proteins. Bcl-2, is also known as B-cell lymphoma 2, and was first identified as an apoptosis.

Inhibitory protein overexpressed in human follicular B-cell lymphomas due to translocation in chromosome number 14 and 18.[38] Following this, three major groups of Bcl-2 family proteins have been identified in mammals. The original group includes Bcl2, Bcl-xL, and Mcl-1, an opposite functional group have also been found, which is also called pro-apoptotic BH123 protein group which includes Bax and Bak; and the third group which is called apoptosis initiator group is made of BH3 domain-proteins, including Bad, Bid, Bim, Puma, and Noxa. When there are no apoptotic stress, Bcl-2, and Bcl-xL (pro-survival) form heterodimers with Bax and Bak (proapoptotic) and hence maintains the integrity of OMM and stops mitochondrial apoptosis. In the presence of factors which stimulate apoptosis, the expression of proapoptotic proteins Bax and BH3-proteins (apoptosis initiator) increases, after that they bind to pro-survival Bcl-2 proteins to release Bax/Bak. Free Bax and Bak form oligomers, which lead to the release of cyt c from the intermembrane space of mitochondria to the cytosol, by forming a channel in OMM. The cyt c which is released activates the caspase cascade to induce apoptosis.[39] To understand the function of Bcl-2 protein *in vivo*, numerous mouse models have been developed. Loss of Bcl-2 in mouse results in numerous defects such as, delayed growth, decreased life span, polycystic kidney, atrophy in thymus, and spleen.[40] Bcl-2 null mice also show some defects in neurons during the neonatal period.[41] Similarly, mice not having Bcl-xL show early embryonic lethality due to the excess apoptosis of neurons in the brain, spinal cord, and erythroid cells in the liver, which indicates the role of Bcl-xL during neuron and erythrocyte maturation.[42] This data strongly support the inhibitory function of Bcl-2 and Bcl-xL in apoptosis, though the function may be tissue and developmental stage specific. The deletion of any one BH3-only gene in mice, does not result in any significant developmental defects,[1] although Bid deletion inhibits Fas-induced apoptosis in some cell types.[43] However, mice with Bid, Bim, and Puma did not showed any embryonic lethality, and a subset of the viable triple null mice showed similar developmental defects to those of Bax-/-Bak-/-mice with interdigital webs of skin on their feet and imperforate vaginas, indicating that, these three BH3-only proteins in combination are essential for Bak/Bax activation.[44]

ROLE OF CYTOCHROME C IN DIFFERENT DISEASES

Many neurodegenerative diseases are characterized by the loss of specific neurons due to the induction of apoptosis. The impairment in the function of mitochondria leads to the release of cyt c into the cytosol following apoptosis, has been demonstrated in acute neurologic trauma such as stroke[45] and brain and spinal cord injury due to any trauma[46] as well as chronic diseases such as amyotrophic lateral sclerosis (ALS),[47] Huntington's disease,[48] and Parkinson disease.[49] If the release of cyt c could be inhibited, apoptosis could be stopped, slowing down the disease progression, and limiting neurologic damage after trauma. Reperfusion of tissues in the brain after ischemia initiates a cell death cascade which exhibits hallmarks of our proposed cyt c-centered mechanism which also includes altered mitochondrial Ca^{2+} homeostasis,[50] Ca^{2+}-dependent dephosphorylation of proteins in mitochondria, more number of ROS generation, and cardiolipin peroxidation and redistribution,[51] all ending in the release of cyt c from mitochondria and inducing apoptotic cell death. Many studies have shown that therapeutic intervention at the level of cyt c release is an effective neuroprotective method,[52] and that the cyt c release is required for apoptosis to occur.[53] Similar types of injury have been demonstrated following traumatic injury to the brain[54] and spinal cord,[55] which demonstrates a central role of cyt c release in acute neurologic trauma. Familial ALS, which is a chronic neurodegenerative disease, is characterized by the presence of high levels of ROS due to mutations in the radical scavenger superoxide dismutase.[56] An increase in the level of ROS damage cells, which results in the loss of specific motor neurons through apoptosis. Cardiomyopathy which is mostly contributes to congestive heart failure and is initiated by apoptosis of cardiac muscles. Immunogold labeling and immunoblotting of cardiomyopic hearts with

anti-cyt c and anticaspase 3 antibodies showed significant amount of release of cyt c into the cytoplasm and activation of caspase 3 when compared to normal heart.[57] Cardiac ischemia follows a similar pathway with sudden reperfusion causing excessive ROS production which leads to cyt c release and ultimately apoptosis. Cyt c release in cardiac muscles has been attributed to many mechanisms including ROS generation, cardiolipin peroxidation, and Ca^{2+} overload in the mitochondria. Indeed, compounds which prevent cardiolipin peroxidation (melatonin) protect mitochondria exposed to Ca^{2+} overload[58] and limit reperfusion injury in the heart.[59] Further studies, in particular, the explanation of the signaling pathways which leads to reversible phosphorylation of cyt c, which is necessary to determine the contribution of cyt c phosphorylation in the regulation of cell death. This knowledge may help in the development of therapies that target cyt c directly or through cell signaling pathways to prevent stress stimuli from damaging which is otherwise the functional cell population. Furthermore, conditions in which increased apoptotic activity would be beneficial as it can be targeted, such as cancer. A general problem of controlling cancer are adaptive mechanisms that allow cancer cells to escape the apoptotic pathway, and cyt c (hyper-) phosphorylation or the inability to dephosphorylate cyt c may be one of the underlying mechanism.

CONCLUSION

Cyt c is one of the most intensively studied proteins. The involvement of cyt c in several processes which is crucial for life and death of cells, including electron transfer, redox-coupled protein import, cardiolipin oxidation, scavenging of free radicals, and the apoptosome formation, make it a like target of regulation by posttranslational modifications. The role of these phosphorylation to control respiration rates and the production of ROS, in addition to direct regulation of cell-death-associated processes such as cardiolipin oxidation and the apoptosome formation open up the possibility for future manipulation of cyt c phosphorylation in pathological conditions where decreased or in contrast, increased apoptotic activity would be beneficial, such as neurodegenerative diseases and cancer, respectively.

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Conflicts of interest
There are no conflicts of interest.

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