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Reduction of ER-Mitochondria Distance: a Key Feature in Alzheimer's and Parkinson's Disease, and During Cancer Treatment

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Abstract— **One remarkable dynamic cell structure is the region between the endoplasmic reticulum (ER) and the mitochondria, termed the mitochondria-associated membranes (MAM). MAMs carry out different cellular functions such as Ca^{2+} homeostasis and lipid synthesis, which depend on an adequate distance separating the ER and mitochondria. A decreased distance has been observed in Alzheimer's disease, Parkinson's disease, and during cancer treatment. It is unclear how dysregulation of the spatial characteristics of MAMs can cause abnormal Ca^{2+} dynamics which could end in cell death. In this work, a computational model was proposed to study the relationship between a decreased ER-mitochondria distance and mitochondria-induced cell death. Our results point towards the mitochondrial permeability transition pore (mPTP) as a key cell death signaling mechanism indirectly regulated by the spatial characteristics of MAMs.**

Clinical Relevance— **The endoplasmic reticulum-mitochondria crosstalk plays an important role in the mPTP-induced apoptosis. This process could be behind neurodegeneration in Alzheimer's and Parkinson's diseases, as well as behind the induced cell death during cancer treatment.**

I. Introduction

One common feature among Alzheimer's disease, Parkinson's disease and cancer is the dysregulation in spatial characteristics of the mitochondria-associated membranes (MAMs)[1], [2]. MAMs are specific regions where the endoplasmic reticulum (ER) is in close contact with the mitochondria (Mt). These house more than 1000 proteins and provide a platform that is fundamental for several cellular functions, such as Ca^{2+} homeostasis, autophagy, lipid metabolism and apoptosis.

MAMs are involved in the transport of Ca^{2+}, an important second messenger involved in different biological processes including contraction of muscles, metabolism, cell proliferation, steroid synthesis, and gene expression. In cells, the main reservoir of this ion is the ER. When there is an extracellular stimulus such as a hormone, the cell responds by internally producing inositol 1,4,5-trisphosphate (IP_3). IP_3 diffuses in the cytosol and binds to the inositol 1,4,5-trisphosphate receptor (IP_3R) which in turn triggers the release of Ca^{2+} from the ER. The proximity between the ER and Mt allows the Mt to take up Ca^{2+} to help fuel the oxidative metabolism and create the proton gradient that maintains the mitochondrial membrane potential (V_m) necessary for ATP production [3].

Ca^{2+} signaling depends on the ER-Mt distance, normally around 20-40 nm [4]–[6]. In Alzheimer's disease, Parkinson's disease, and during chemotherapy periods for cancer treatment, a shorter than normal width of MAMs (<15 nm) has been shown to contribute to a higher transfer of Ca^{2+} into the Mt and an excessive cytosolic Ca^{2+} concentration. The high mitochondrial Ca^{2+} concentration can cause the depolarization of the V_m which could lead to the prolonged opening of the mitochondrial permeability transition pore (mPTP) [7]–[10].

The mPTP is a key mechanism of cell death signaling and under normal conditions it is in its low-conductance state, i.e. the flow of ions (such as Ca^{2+}, H^+ and Mg^{2+}) and small solutes through this channel is very small. In this conductive state of the mPTP, V_m is maintained at high voltages (150-190 mV) [11]. However, in pathological conditions, it is hypothesized that MAMs' shorter width causes a high mitochondrial Ca^{2+} concentration and a strong depolarization of the V_m which could trigger the permanent high-conductance state of mPTP. If mPTP opens fully, there could be a big flow of ions and solutes leading to a complete V_m dissipation, increased generation of reactive oxygen species, decreased ATP production, and ultimately start the apoptotic process observed in Alzheimer's disease, Parkinson's disease and during cancer treatment [12].

In this work, we proposed a Ca^{2+} signaling model to study the effect of a shorter distance between the ER and Mt on Ca^{2+} dynamics and its possible link to the opening of the mPTP in order to analyze the associated Ca^{2+} dynamics and V_m, which in turn could lead to cell death.

II. Methods

A. Model for ER-Mt distance and mPTP

Qi et al. [13] proposed a model that describes the Ca^{2+} dynamics within the ER-Mt region as a function of the distance separating both organelles. Qi's model considers four compartments: cytosol (Cyt), ER, Mt, and microdomain (Mic). The region of proximity between the ER and Mt is

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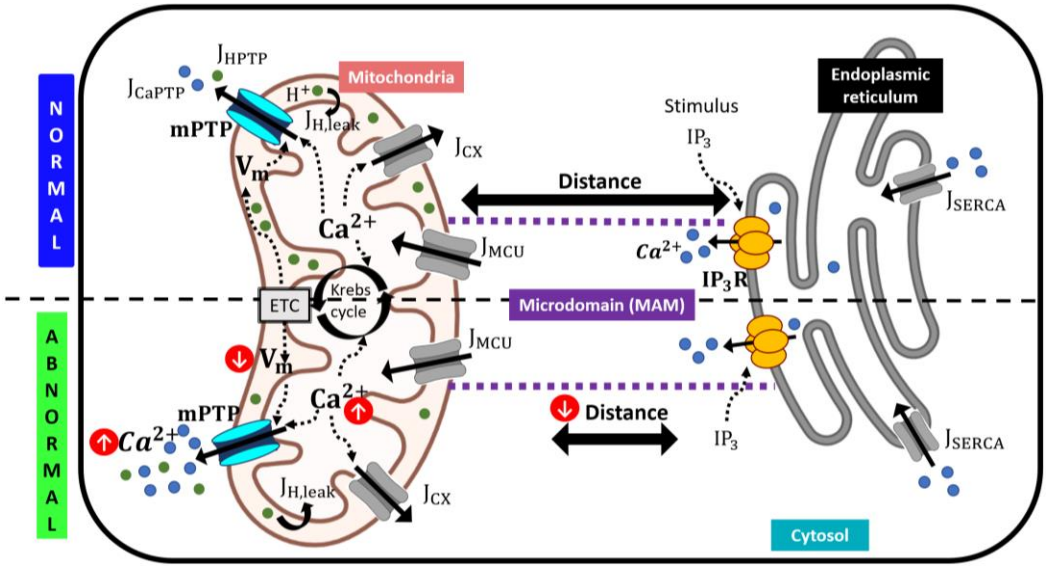

Figure 1. Proposed model to study the relationship between a reduction of ER-mitochondria distance and the key cell death mechanism, mPTP. In response to an extracellular stimulus, the cell produces internally 1,4,5-trisphosphate (IP_3) which triggers the release of Ca^{2+} from the endoplasmic reticulum (ER) through the inositol 1,4,5-trisphosphate receptor (IP_3R). Due to the proximity of ER and mitochondria, the mitochondria can sense the high Ca^{2+} concentration (microdomain) created by the IP_3Rs. The Ca^{2+} uptake by the mitochondria (Mt) is mediated by the mitochondrial Ca^{2+} uniporter (J_{MCU}) and the extrusion by Ca^{2+} exchangers (J_{CX}). Mitochondrial Ca^{2+} participates in the Krebs Cycle allowing the generation of a proton gradient by the electron transport chain to establish the mitochondrial membrane potential (V_m). A proton leakage into the mitochondrial matrix is considered ($J_{H,leak}$). In normal conditions of a proper ER-Mt distance (top half), the mitochondrial Ca^{2+} helps maintain a high V_m and the permeability transition pore (mPTP) is practically closed. In this low-conductance state of the mPTP, the flux of Ca^{2+} (J_{CaPTP}) and other ions (J_{HPTP}) is small. In abnormal conditions of a decreased ER-Mt distance (bottom half), Ca^{2+} accumulates in the Mt, the V_m dissipates and mPTP completely opens releasing large quantities of Ca^{2+} into the cytosol.

modeled as a Mic, a section of local high cytosolic Ca^{2+} concentration compared to the bulk cytosolic concentration (10-30 µM vs 1-3 µM, respectively)[14]. However, this model does not consider the mitochondrial regulation that could lead to cell death.

Wacquier et al. [12] proposed a model that describes the mitochondrial Ca^{2+} dynamics and the key mechanism involved in the initial process of cell death, the mPTP. It provides a more rigorous description of the Mt that includes the participation of Ca^{2+} in Krebs Cycle and the oxidation of NADH in the electron transport chain which helps maintain the V_m. The model can reproduce the experimentally observed positive feedback of mitochondrial Ca^{2+} on V_m and how the V_m, in turn, affects the opening of the mPTP.

In order to analyze how the decreased ER-Mt distance affects the opening of the mPTP, we propose to model ER and Mic processes with Qi's model and Mt processes with Wacquier's model.

A schematic representation of the proposed spatio-temporal model is shown in Fig. 1. The influx of Ca^{2+} into the ER is mediated by the sarcoendoplasmic reticulum Ca^{2+}-ATPase (J_{SERCA}). The signaling molecule, IP_3 binds to IP_3R and evokes an ER Ca^{2+} release (J_{IP3R}) into the Mic. Ca^{2+} in the Mic is sensed by the mitochondrial calcium uniporter (J_{MCU}), which along with the mechanism of calcium extrusion (J_{CX}) helps regulate mitochondrial Ca^{2+} concentration (Ca_{Mt}). The flux of Ca^{2+} into the Mt participates in the Krebs Cycle reducing NAD^+ to NADH. NADH is then oxidized (J_o) in the electron transport chain to feed protons into the inner mitochondrial membrane and create the gradient necessary for the V_m. A proton leak (J_{Hleak}) that is proportional to V_m is considered. The membrane potential and the mitochondrial Ca^{2+} regulate the opening of the mPTP. In the low conductance state (normal conditions), the flux of protons and Ca^{2+} (J_{HPTP} and J_{CaPTP}) is low but at high mitochondrial Ca^{2+} concentrations (abnormal conditions), the mPTP fully opens and fluxes J_{HPTP} and J_{CaPTP} are big. The dynamic equation describing the temporal evolution of V_m is:

$$dV_m/dt = (a_1 J_o - J_{Hleak} - J_{CX} - 2J_{MCU} + 2J_{CaPTP} - J_{HPTP})/C_p \quad (1)$$

a_1 scales NADH consumption into voltage variations and C_p is the membrane capacitance. The differential equation describing the fraction of open mPTP is:

$$dPTP/dt = V_{op}(1-PTP)\,(1+\exp((V_m - q_{op}Ca_{Mt})/q_{11})^{-1} - V_{cl}\,PTP \quad (2)$$

V_{op} and V_{cl} describe the rate constants of mPTP opening and closing, whereas the opening of the mPTP dependent on Ca^{2+} and voltage are given by q_{op} and q_{11}. A small PTP value represents the low-conductance state and a high value the high-conductance state.

TABLE I. VALUES OF FITTED PARAMETERS FOR MODEL

Parameter	Definition	Value
V_{MCU}	Rate constant of the MCU	5.40 x 10^{-6} µM/s
V_{CX}	Rate constant of the Ca^{2+} exchangers (J_{CX})	1.09 µM/s
V_{CaPTP}	Rate constant of Ca^{2+} flux through mPTP	10 µM/s
V_{HPTP}	Rate constant of protons flux through mPTP	67500 µM/s
q_{op}	Voltage and mitochondrial Ca^{2+} dependence coefficient of mPTP opening	2.40 mV/µM

For the proposed model, all the corresponding parameters in [12] and [13]were used with the exception of the fitted parameters shown in Table 1. V_{MCU}, V_{CX} and V_{CaPTP} were fitted to be able to replicate physiological Ca^{2+} oscillations in each compartment [15], [16]. Values for V_{HPTP} and q_{op} established the dependence of high and low-conductance state of the mPTP on Ca_{Mt} that has been observed experimentally for normal conditions [8], [11], [12]. Simulations were obtained using Matlab R2020a built-in function ode15s with the following initial conditions: ER Ca^{2+} concentration = 405.04 μM, Mt Ca^{2+} concentration = 0.04 μM, Cyt Ca^{2+} concentration = 0.10 μM, V_m = 192.10 mV and PTP = 2.36 x 10^{-4} . Initial conditions were calculated at steady state when ER-Mt distance is 35 nm and IP_3 concentration is 0.01 μM. These values correspond to physiological basal conditions and were used for all simulations [11], [13].

III. RESULTS & DISCUSSION

A. Effect of ER-Mt distance on mPTP opening

In Fig. 2 the temporal evolution of Ca^{2+} in the Mt and Cyt, V_m, and the fraction of open mPTP is shown under normal conditions i.e., at an ER-Mt distance of 20 and 40 nm (red and blue traces) and at a pathological distance of 10 nm (green trace).

As observed in these simulations, at normal distances (red and blue traces), when the cell is stimulated with an IP_3 concentration of 0.5 μM, there is an initial high Ca^{2+} peak in the Mt that then settles into a stable oscillatory regime at around 50 μM (see Fig. 2A). In response to Ca_{Mt} variations, there is a transitory V_m depolarization that can reestablish back to normal levels at around 180 mV as observed experimentally (see Fig. 2B) [11]. This allows the mPTP to remain practically closed or in the low-conductance state (see Fig. 2C) and the cytosolic Ca^{2+} concentration (Ca_{Cyt}) oscillation to be around 2.5 μM with the amplitude and frequency as experimentally observed [15], [16].

In contrast, simulations at an abnormal distance of 10 nm (green trace in Fig. 2) show that the first elevated mitochondrial Ca^{2+} peak (Fig. 2A) together with the lowering of V_m provoke the opening of the mPTP in its high-conductance state (Fig. 2B). Then, the permanent opening of the mPTP (Fig. 2C) causes the release of a great amount of Ca^{2+} into the Cyt. This Ca^{2+} overload is directly associated with cell death [2], [8], [10]. Ca_{Cyt} now oscillates faster and with a higher concentration of approximately 5 μM (Fig. 2D). This behavior results in a 100% increase in Ca^{2+} concentration compared to the normal conditions along with an increase in frequency. The long-lasting opening of the mPTP occurs for distances < 12 nm.

Our results agree with what has been observed in Alzheimer's disease, a neurodegenerative disease characterized by the massive death of neurons and extracellular formation of amyloid beta plaques (aggregates of protein). Familial Alzheimer's disease has been linked to genetic mutations that shorten the ER-Mt distance, causing a higher Ca^{2+} transfer into the Mt, Ca_{Cyt} accumulation, and high-amplitude and high-frequency intracellular Ca^{2+} concentrations believed to

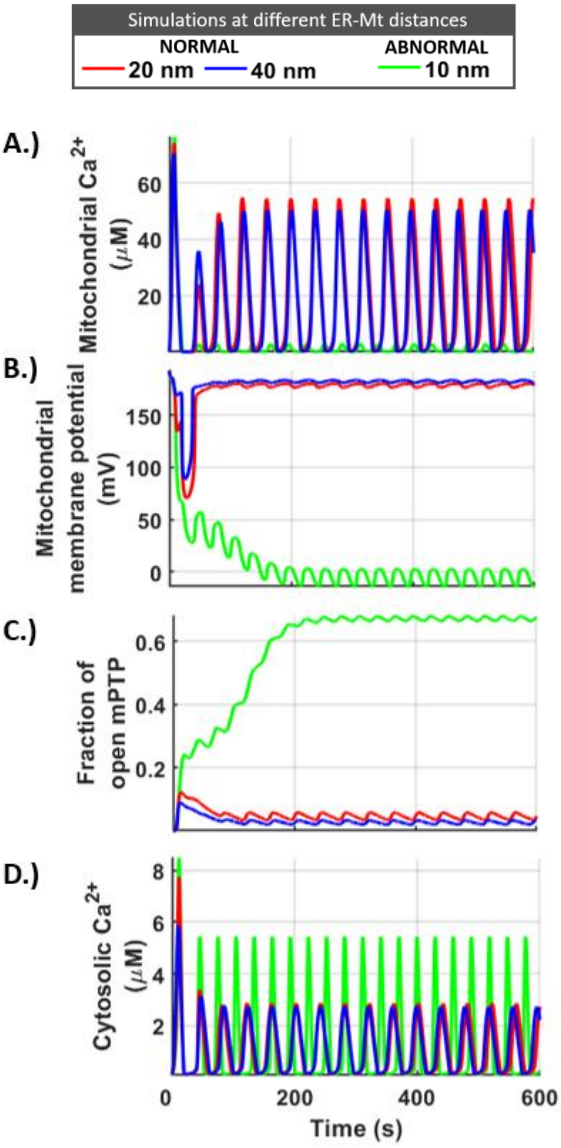

Figure 2. A reduction of ER-Mt distance opens the key cell death mechanism, mPTP. Computational simulations of the effect of the ER-Mt distance on A.) mitochondrial Ca^{2+}, B.) mitochondrial membrane potential, C) fraction of open mPTP and D.) cytosolic Ca^{2+}. Stimulation with [IP_3] = 0.5 μM at distances of 10 (green), 20 (red), and 40 nm (blue).

be triggered by mPTP opening [6], [9].

Parkinson's disease is also a neurodegenerative disease distinguished by the loss of dopaminergic neurons and generation of protein aggregates called Lewy bodies (analogous to amyloid beta plaques). Similar to Alzheimer's disease, familial Parkinson's disease is related to mutations in nuclear genes encoding α-synuclein that has been reported to participate in the ER-Mt connection. It has been found that there is more Ca^{2+} transfer from the ER to the Mt suggesting that the back-and-forth communication between the two organelles could play an important role in the apoptotic process of this disease [1].

Interestingly, for breast cancer cells, it has been shown that an increased sensitivity to chemotherapy-induced apoptosis

could be linked to a higher presence of decreased ER-Mt distances (approximately less than <30 nm) causing accumulation of Ca_{Mt}, mPTP opening and an increase (58%) in Ca_{Cyt} [2], [17]. It is worth mentioning that in this work authors only reported ER-Mt distances above 30 nm. Our finding (<12 nm) belongs to this range and points toward the need for future experimental assessment of distances with more spatial resolution.

All these reported observations could be related to our results since we also obtained higher Ca^{2+} concentrations and faster Ca^{2+} oscillations when ER-Mt distance is reduced.

IV. Conclusions

In this work, the effect of reducing the distance between the ER and Mt on the opening of a key mechanisms that signals cell death was studied using a computational model. Our model is able to predict that as the distance decreases there is a permanent opening of the mPTP (for distances < 12 nm.), as has been hypothesized for Alzheimer's disease, Parkinson's disease, and during cancer treatment. This work highlights the importance of the spatial characteristics of the ER-Mt connection and their intercommunication to keep the proper Ca^{2+} homeostasis, as well as the implications of dysregulation.

Our model is primarily designed for non-excitable cells. It should be noted that we consider that the outer mitochondrial membrane is highly permeable and that there are no adenine nucleotides, powerful inhibitors of mPTP [12]. At the same time, the ER Ca^{2+} is dynamic but the IP_3R Ca^{2+} release is assumed to be a point source and the spatial characteristics among IP_3Rs are not considered. Therefore, our model would have to be adapted to provide relevant simulations for specific clinical applications. For example, our model could be used to further study the intracellular conditions behind other diseases or conditions such as amyotrophic lateral sclerosis [7], obesity, postprandial metabolism [18], and to analyze the use of synthetic linkers to improve symptoms [4] in order to better understand how cells might modulate the ER-Mt distance to compensate for cellular dysregulations and how to ameliorate abnormal conditions.

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