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Cdk5 Loss Alters Mitochondrial Cristae Organization

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Mitochondria are key cellular organelles executing diverse fundamental functions, including energy conversion, calcium homeostasis, immunity and cell death. These roles are intricately regulated by changes in the structural morphology of the mitochondria[1]. These changes include mitochondrial fusion and fission that are controlled by the multidomain GTPase dynamin-related proteins (DRPs): mitofusin 1/2 (Mfn1/2), optic atrophy 1 (Opa1) and dynamin-related proteins 1 (Drp1)[1]. Mfn1/2 and Opa1 promote fusion of adjacent mitochondria while Drp1 stimulates fission. Mfn1/2 is required for the initial tethering of fusing mitochondria[2, 3]. Physical interaction of Mfn1/2 with Opa1, which is stabilized by an assembly of OPA1 in the intermembrane space (IMS)[4], then allows mitochondrial fusion. Conversely, Drp1 causes fission by forming distinct spiral structures on the mitochondrial outer membrane[1]. Mitochondrial fission is also regulated by posttranslational modification of Drp1. Phosphorylation of Drp1 at serine 616 (Ser_{616}) by extracellular signal-regulated kinase 2 (Erk2) promotes fission[5] while phosphorylation at serine 637 (Ser_{637}) by protein kinase A (PKA) inhibits the process[6]. In addition, dephosphorylation of Drp1 at Ser_{637} by calcineurin stimulates fission[7].

Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine kinase that was discovered based on its sequence identity to the key cell cycle regulators, Cdk1 and Cdk2[8]. However, Cdk5 belongs to the atypical Cdks whose roles are not directly linked to cell cycle control[8]. Recently, Cdk5, a known cytoplasmic protein, was localized to mitochondria, specifically at the inner membrane[9], and such localization has been associated with a role in regulating mitochondrial dynamics[7]. For example, we recently demonstrated that loss of Cdk5, including mitochondria, causes mitochondrial permeability transition pore (mPTP) opening and subsequent depolarization as well as mitochondrial fission and ROS increase[7]. These events lead to decreased ATP production and ultimately apoptosis in breast cancer cells[7]. Treatment with an mPTP inhibitor, cyclosporine A or sanglifehrin A, reverses the phenotypic effects, including breast cancer cell apoptosis, due to Cdk5 loss[7], supporting a role for Cdk5 in regulating mPTP dynamics.

Maintenance of structural integrity and balance between fusion and fission states are crucial for proper mitochondrial function; perturbation of which could trigger apoptosis. Increased fission resulting from DRP1 translocation into mitochondria and oligomerization at the fission site, causing membrane ingression, is an early apoptotic event[1] that is influenced by increased intracellular

calcium concentration ($[Ca^{2+}]_i$)[7]. In breast cancer cells, we found that Cdk5 loss causes elevated $[Ca^{2+}]_i$ that coincides with a surge in Ca^{2+}-dependent calcineurin activity and subsequent dephosphorylation of DRP1 at Ser_{637} as well as mitochondrial fission[7]. These findings suggest that Cdk5 loss promotes breast cancer cell apoptosis by inducing mitochondrial fission via the $[Ca^{2+}]_i$-calcineurin-DRP1 Ser_{637} dephosphorylation cascade.

Cristae, the oxidative phosphorylation site in mitochondria, are deep invaginations that result from disruption in the continuity of the inner mitochondrial membrane (IMM). The IMM is squeezed at its base into narrow openings called cristae junctions, which prevent diffusion of cristae contents such as cytochrome c into the IMS. Indeed, the release of cytochrome c into the cytoplasm is an early apoptotic event triggered by IMM ultrastructure dynamics or cristae remodelling. However, it was reported that mPTP also regulates cristae junction remodelling for cytochrome c-dependent apoptosis induced by ER stress[10]. In previous studies, we further found that loss of Cdk5, which regulates mPTP dynamics[7], results in caspase activation[7] that is mediated by cytochrome c release, further supporting a role for Cdk5 in mitochondria-mediated apoptosis, and potentially, in cristae organisation.

Opa1 controls cristae integrity independently of its role in mitochondrial fusion[11]. In humans, eight alternatively spliced variant forms of Opa1 encode for proteins that contain N-terminal mitochondria targeting sequence, a transmembrane and a coiled-coil domain[11]. Cleavage of the mitochondrial targeting sequence generates the membrane-anchored Opa1 long forms (L-forms) that are further cleaved at their N terminus by Opa1 proteases,

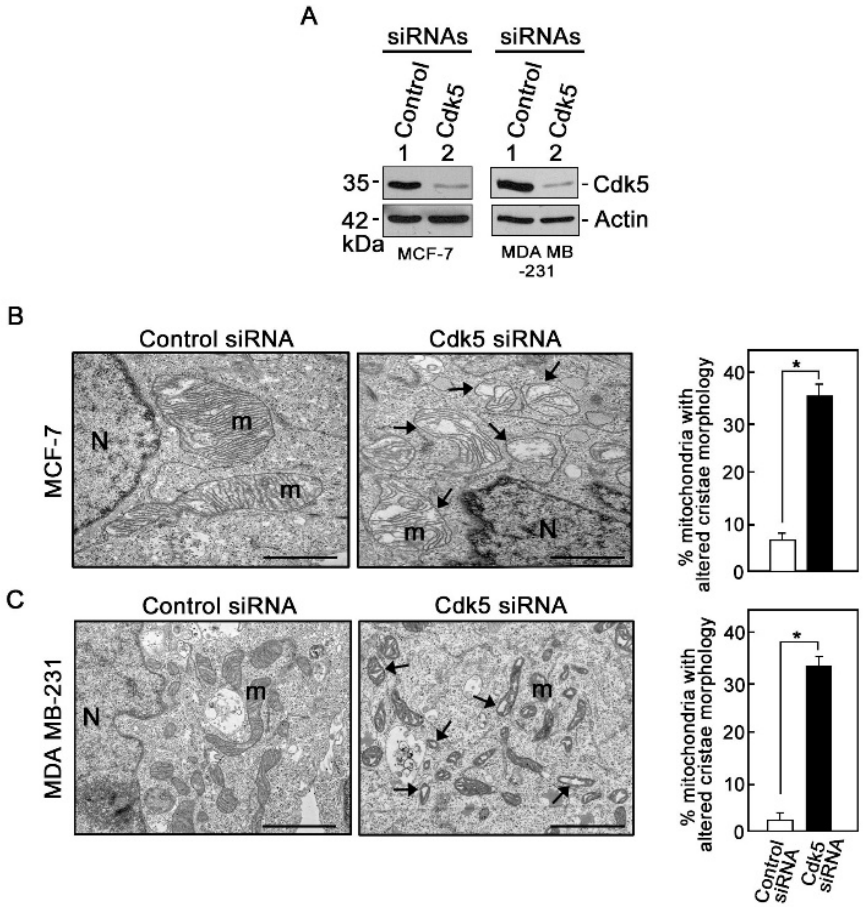

Figure 1. Loss of Cdk5 causes mitochondrial cristae disorganization in breast cancer cells. A. MCF-7 and MDA MB-231 breast cancer cells transfected with Cdk5 siRNA (100 nM) for 72 hrs were lysed and subjected to immunoblotting for Cdk5. Actin blot serves as loading control. Blots shown represent one of three sets of blots with similar patterns. B and C. Transmission electron microscopy (TEM) images of mitochondrial ultrastructure in MCF-7 (B) and MDA MB-231 (C) cells transfected with control or Cdk5 siRNA. Images were acquired using Hitachi H7650 TEM at 7,000x magnification. Scale bar = 500 nm. Arrows in Cdk5-depleted cells are directed at mitochondria (m) with altered cristae structure. N represents nucleus. Graphs show the % of mitochondria with clearly disorganized cristae structure. Data were acquired by blindly analyzing 50 mitochondria per treatment group in each of three independent experiments. Values are means ± SEM from the three independent experiments. * indicates $p<0.05$ using student's t-test (unpaired).

generating the soluble short forms (S-forms) in the IMS[11]. Oligomerization of S- and L-forms in the IMS and the IMM, respectively, regulates the formation of cristae junctions. Previously, it was shown that Cdk5 interacts with and phosphorylates Omi/HtrA2[9], an Opa1 protease[12]. Specific phosphorylation of Omi/HtrA2 at Ser_{400} by Cdk5 elevates its protease activity[9], increasing Opa1 cleavage and thus cristae junction formation[12]. Indeed, defects in Opa1 processing have been shown to cause disorganized cristae structure[13]. Since OPA1 is also cleaved by OMA1 and Yme1L, it would be interesting to examine whether loss of Cdk5 affects the activity of OMA1 and Yme1L.

Cristae organisation is also regulated by $[Ca^{2+}]_i$[14]. It is interesting that depletion of OPA1 was shown to increase the rate of mitochondrial Ca^{2+} uptake, indicating the importance of OPA1 in regulating mitochondrial Ca^{2+} dynamics[15], and suggesting a role for $[Ca^{2+}]_{mt}$ in controlling OPA1-mediated cristae organisation. In separate studies, Ca^{2+} transfer from the endoplasmic reticulum (ER) to the mitochondria and subsequent activation of mPTP was also found to contribute to cristae remodelling[16]. Thus, our observation that Cdk5 loss in primary mouse embryonic fibroblasts (MEFs) causes increased Ca^{2+} transfer from the ER to the mitochondria, resulting in increased $[Ca^{2+}]_{mt}$ and mPTP opening[17] further supports a link between Cdk5 and cristae organisation.

Here, we sought to examine whether Cdk5 loss triggers a change in IMM topology and affects cristae structural organization. As shown in Figure 1, Cdk5 depletion by siRNA in two representative breast cancer cell lines, MCF-7 and MDA MB-231, and subsequent electron microscopy revealed that while cells transfected with control siRNA displayed normal tubular mitochondria with well-organized cristae, cells depleted of Cdk5 showed irregular cristae structures with increased spaces between adjacent cristae. This dramatic alteration in cristae structure in breast cancer cells depleted of Cdk5 was recapitulated in *ex vivo* MEFs from $Cdk5^{-/-}$ mice (Figure 2). Altogether, our results indicate a novel role for Cdk5 is maintaining mitochondrial cristae integrity and thus loss of Cdk5 causes cristae disorganization. Since Cdk5 phosphorylates Omi/HtrA2 at Ser_{400}[9], increasing its protease activity[9] and Opa1 cleavage[12], it is possible that loss of Cdk5 results in loss of Omi/HtrA2 Ser_{400} phosphorylation and reduced Opa1 cleavage, leading to disorganized cristae structure. Since Cdk5 further regulates $[Ca^{2+}]_{mt}$ and mPTP dynamics,

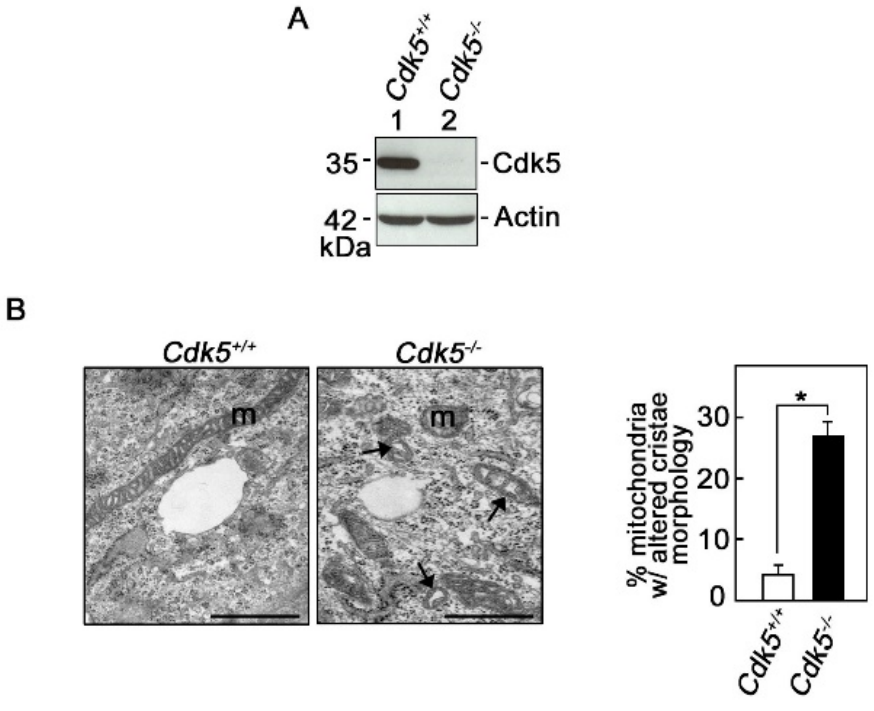

Figure 2. $Cdk5^{-/-}$ primary mouse embryonic fibroblast cells exhibit disorganized cristae structure. A. Lysates of wt and $Cdk5^{-/-}$ MEFs isolated from wt and $Cdk5^{-/-}$ E13.5 embryos were subjected to SDS-PAGE and immunoblotting for Cdk5. Actin blot serves as loading control. Blots shown represent one of three sets of blots with similar patterns. B. TEM of mitochondrial ultrastructure in wt and $Cdk5^{-/-}$ MEFs. Arrows in $Cdk5^{-/-}$ MEFs are directed at mitochondria with disorganized cristae. Images were acquired using Hitachi H7650 TEM at 10,000x magnification. Scale bar = 500 nm. Graph shows the % of mitochondria (m) with clearly disorganized cristae structure. Analysis was performed blindly in ten different fields per treatment group. Values represent means ± SEM from three independent experiments. * indicates $p<0.05$ using student's t-test (unpaired).

which have both been implicated in cristae organization, it is also possible that altered $[Ca^{2+}]_{mt}$ or mPTP opening led to cristae disorganization upon loss of Cdk5. However, a combined effect of Cdk5 loss on Omi/HtrA2 Ser_{400} phosphorylation, $[Ca^{2+}]_{mt}$, and mPTP dynamics likely causes cristae disorganization.

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