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Type 2 Transglutaminase, mitochondria and Huntington's disease: Menage a trois

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ABSTRACT

Mitochondria produce the bulk of cellular energy and work as decisional "hubs" for cellular responses by integrating different input signals. The determinant in the physiopathology of mammals, they attract major attention, nowadays, for their contribution to brain degeneration. How they can withstand or succumb to insults leading to neuronal death is an object of great attention increasing the need for a better understanding of the interplay between inner and outer mitochondrial pathways residing in the cytosol. Of the latter, those dictating protein metabolism and therefore influencing the quality function and control of the organelle are of our most immediate interest and here we describe the Transglutaminase type 2 (TG2) contribution to mitochondrial function, dysfunction and neurodegeneration. Besides reviewing the latest evidences we share also the novel ones on the IF_1 pathway depicting a molecular conduit governing mitochondrial turnover and homeostasis relevant to envisaging preventive and therapeutic strategies to respectively predict and counteract deficiencies associated with deregulated mitochondrial function in neuropathology.

1. Introduction

Transglutaminase type 2 (TG2 or tissue transglutaminase) (Grosso and Mouradian, 2012; Kim et al., 1999) is a multifunctional ubiquitously expressed member of TG (TGs) family that catalyses post-translational modifications of proteins through Ca^{2+} dependent reactions (Fesus and Piacentini, 2002) (Fig. 1A). The multi-functionality of TG2 is dependent on its structural features. The structure of the enzyme, crystallised in a dimer form in a complex with GDP, is composed of 4 domains, a N-terminal β-sandwich domain with fibronectin and integrin binding sites (aa 1–140), the catalytic core containing the catalytic triad for the acyl-transfer reaction (aa 141–460) and two C-terminal β-barrel domains (aa 461–586 and 587–687) (Fig. 1A).

In eukaryotic cells, TG2 is regulated by reversible conformational changes (Fig. 1B) that include Ca^{2+}-dependent activation, which shifts TG2 to the "open" conformation thereby unmasking the enzyme's active centre, and inhibition by GTP, GDP, and ATP, which constrains it in the "closed" conformation (Nurminskaya and Belkin, 2012). The site of transamidating activity is composed of the catalytic triad of cysteine proteases: cysteine 277 (C277), histidine 335 (H335) and aspartate 358 (D358), which are the critical residues for the enzymatic activity.

Although it was initially identified and studied as a typical cytoplasmic protein, TG2 was later described to localise in other compartments, including the nucleus, mitochondria, endolysosomes and the extracellular space (Lorand and Graham, 2003; Malorni et al., 2008; Zemskov et al., 2006; Gundemir and Johnson, 2009; Park et al., 2010).

By its transamidating activity, TG2 is able to incorporate amines into glutamine residues forming a polyamine bond leading to the conversion of the substrate glutamine residue into glutamate (Beninati and Piacentini, 2004). TG2 has also a protein disulphide isomerase as well as isopeptidase activity and can hydrolyse the isopeptide bond. Beyond its main transamidating activity, TG2 presents other several enzymatic functions. Its GTPase activity has been shown to impact signal transduction as well as the ability to bind and hydrolyse GTP distinguishing TG2 from other TGs (Fesus and Piacentini, 2002).

Under normal physiology, TG2 is inactive in high concentration of GTP/GDP and low Ca^{2+} levels. GDP binding thus leads to a closed conformation of TG2 that reduces the affinity of the enzyme for Ca^{2+} becoming catalytically inactive (Fig. 1B). However, major changes in

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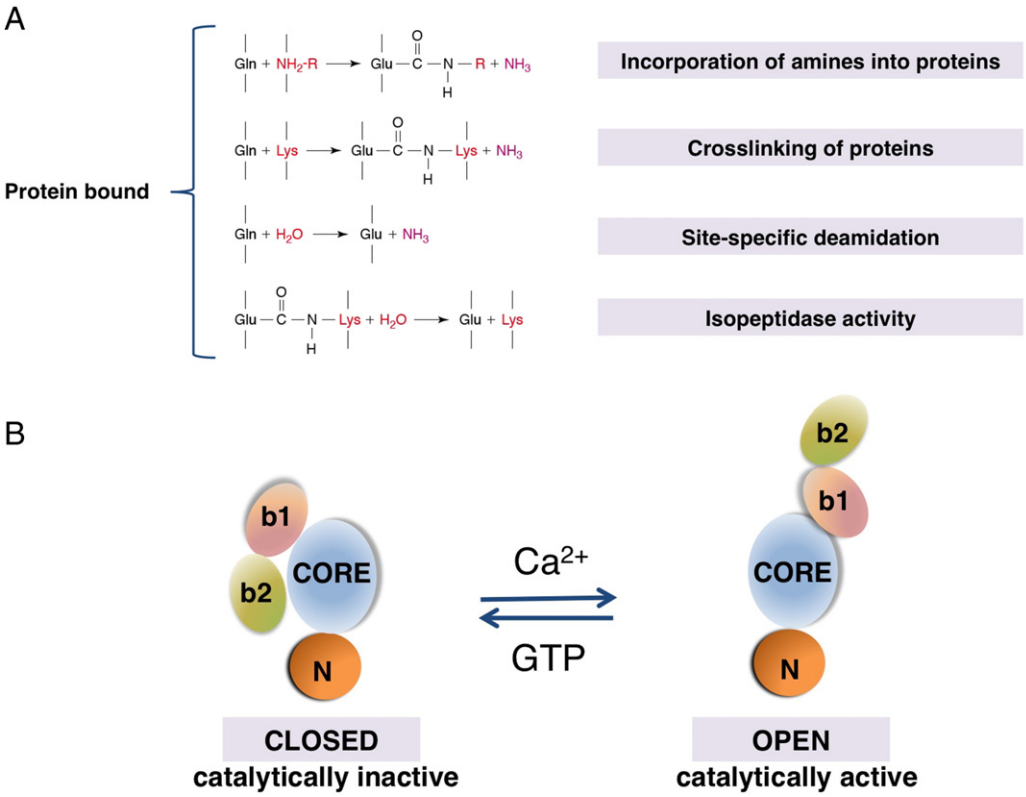

Fig. 1. A) Transamidation and deamidation mechanisms of tissue transglutaminase. B) Ca^{2+} and guanine nucleotide binding inversely regulate the transamidating activity of TG2. GTP bound TG2 has a closed conformation and it is catalytically inactive. Binding of Ca^{2+} is essential to acquire a catalytically active 'open' or 'extended' conformation.

cellular homeostasis leading to the increase of Ca^{2+} in the cytoplasm shift the enzyme towards an open active conformation (Klöck et al., 2012) (Fig. 1B). Recently we focussed our attention on the impact of TG2 expression level on mitochondrial homeostasis and highlighted its relevance to preserving quality of the organelle by manipulating mitochondrial autophagy and its ability to interplay with core regulatory pathways of mitochondrial respiration. Tightly linked to basic energy-dependent functions as well as being associated with more specialised cellular activities, mitochondria undergo active turnover dictated by the autophagy-related events.

We shall summarise the available data and present unpublished ones that will sustain the TG2 fine-tuning role in mitochondria by impacting the core of organelle bio-energy and the upstream regulation of cell mitophagy. This will be done by linking TG2 to the molecular physiology of IF_1, the endogenous regulator of the F_1Fo-ATP synthase, and showing how this is disrupted when TG2 is ablated. Specific evidences will be given on mitochondrial morphology, membrane potential ($\Delta\Psi_m$) and reactive oxygen species (ROS) framing a functional interplay that lies at the heart of cellular homeostasis influencing mitochondrial physiology and quality control.

2. TG2-dependent regulation of the mitochondrial ADP/ATP exchange activity

Almost a decade ago, Hasegawa and his co-workers found that TG2 has relatively low but detectable protein disulphide isomerase (PDI) activity. This activity does not require the presence of either Ca^{2+} or GTP (Hasegawa et al., 2003). They showed that TG2 converted completely inactive RNAase molecules to the native active enzyme that requires free sulfhydryl groups of the protein for catalysis. An interesting role for the PDI activity of TG2 came by analysing TG2 knockout ($TG2^{-/-}$) mice which exhibit glucose intolerance due to impaired glucose induced insulin secretion after intraperitoneal glucose loading (Bernassola et al., 2002). It is also known that impaired glucose-induced insulin secretion often results from impaired glucose-induced ATP elevation or from defective respiratory chain (Fujimoto et al., 2007). Based on these observations, our group provided the first in vivo evidences showing that TG2 acts as PDI and contributes to the correct assembly of the respiratory chain complexes (Mastroberardino et al., 2006). Thus, $TG2^{-/-}$ mice display impairments in mitochondrial energy production and decreased ATP levels after physical challenge. The molecular mechanism behind this phenotype may reside in the defective disulphide bond formation in ATP synthase complex and other key components of the respiratory chain after TG2 deletion (Mastroberardino et al., 2006; Battaglia et al., 2007; Sarang et al., 2009). Additionally, it was found that mitochondrial ADP/ATP transporter adenine nucleotide translocator 1 (ANT1) was incorrectly assembled and was dysfunctional in the absence of PDI activity of mitochondrial TG2 (Malorni et al., 2009). ANT1 is the most abundant protein in mitochondria involved in ADP/ATP exchange across the mitochondrial inner membrane (Liu and Chen, 2013). It serves as a core component of the permeability transition pore complex in the inner mitochondrial membrane (IMM) and also mediates basal proton leak. ANT1 mutations cause severe human diseases including early-onset diseases (Vartiainen et al., 2014). Mice lacking TG2 displayed an increased thiol-dependent ANT1 oligomer formation as well as higher ADP/ATP exchange activity of ANT1 in heart mitochondria. Thereby, PDI activity of TG2 decreased oligomer formation of ANT1 and inhibited its transporter activity by directly binding to ANT1 and sequestering its monomers. These data correlate with current experiments, carried out in our laboratory, showing a higher proton leak, paralleled by augmented oxygen consumption, in cell knockouts for TG2 suggesting that higher respiration rate is required to maintain equal membrane potential (Fig. 2). These findings showed that TG2's