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Article

Dual Proteolytic Pathways Govern Glycolysis and Immune Competence

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SUMMARY

Proteasomes and lysosomes constitute the major cellular systems that catabolize proteins to recycle free amino acids for energy and new protein synthesis. Tripeptidyl peptidase II (TPPII) is a large cytosolic proteolytic complex that functions in tandem with the proteasome-ubiquitin protein degradation pathway. We found that autosomal recessive *TPP2* mutations cause recurrent infections, autoimmunity, and neurodevelopmental delay in humans. We show that a major function of TPPII in mammalian cells is to maintain amino acid levels and that TPPII-deficient cells compensate by increasing lysosome number and proteolytic activity. However, the overabundant lysosomes derange cellular metabolism by consuming the key glycolytic enzyme hexokinase-2 through chaperone-mediated autophagy. This reduces glycolysis and impairs the production of effector cytokines, including IFN-γ and IL-1β. Thus, TPPII controls the balance between intracellular amino acid availability, lysosome number, and glycolysis, which is vital for adaptive and innate immunity and neurodevelopmental health.

INTRODUCTION

Protein degradation occurs continuously within cells. This removes misfolded or damaged proteins and generates free amino acids for protein synthesis or energy production via glutaminolysis (Schutz, 2011). Mammalian cells utilize two principal pathways: proteasomes, which are protein complexes that recognize and degrade ubiquitinated proteins within the cytosol, and lysosomes, which are membrane-bound organelles containing acid hydrolases that are fed substrate by endosomal and autophagic vesicles (Ciechanover, 2005). Evidence suggests that these pathways can cross-compensate to maintain balanced proteolysis and amino acid homeostasis (Korolchuk et al., 2010). In both pathways, proteins are first degraded into long oligopeptides from which N-terminal tripeptides are then trimmed by tripeptidyl peptidases (TPP). These tripeptides are further cleaved by dipeptidyl peptidases and aminopeptidases to generate free amino acids (Tomkinson, 1999).

There are two types of TPP in eukaryotic cells, TPPI and TPPII. TPPI is a lysosomal acid protease, whereas TPPII is a cytosolic protease that forms a giant multisubunit complex acting downstream of proteasomes (Schönegge et al., 2012; Tomkinson, 1999). By trimming long oligopeptides, TPPII was thought to be principally important in producing antigenic peptides that bind to major histocompatibility complex (MHC) class I molecules for presentation to CD8 T cells (Reits et al., 2004). However, the development and function of CD8 T cells was

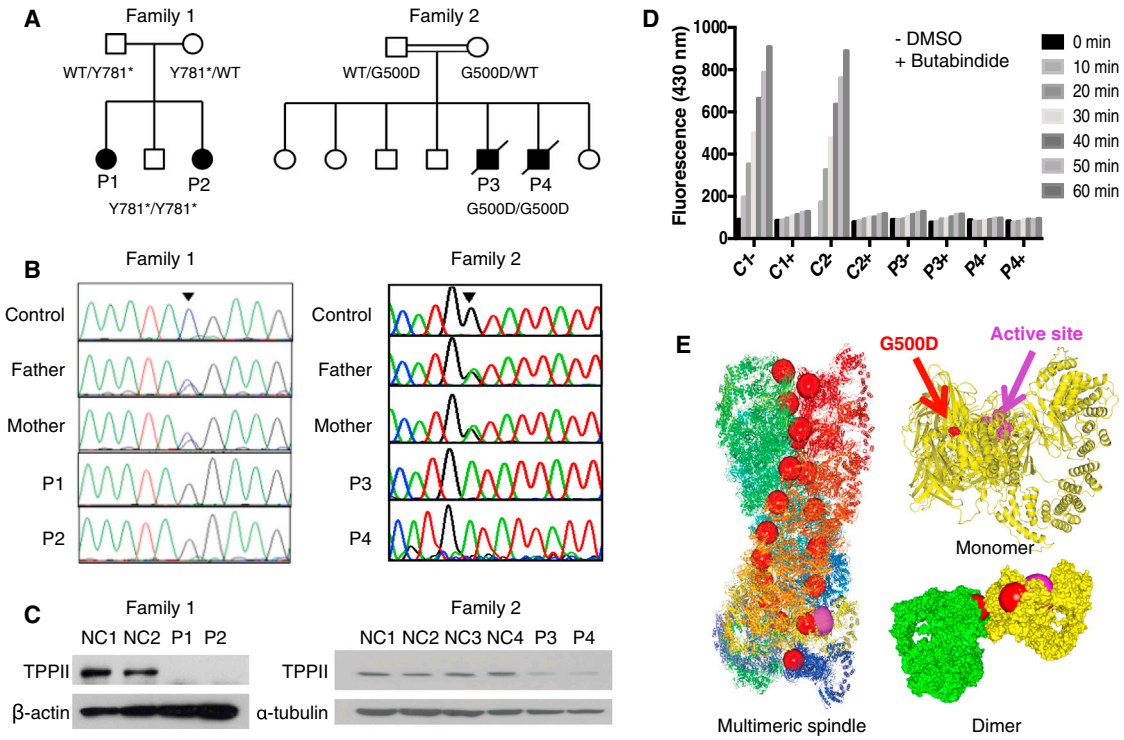

Figure 1. Autosomal Recessive Loss-of-Function Mutations in Human TPPII Deficiency

(A) Patients' pedigrees.

(B) Sanger sequencing showing the mutations.

(C) Immunoblots for TPPII in T cells from P1 and P2 (left) or fibroblasts from P3 and P4 (right).

(D) TPPII enzymatic activity in fibroblast lysates from two healthy controls, P3 and P4, incubated for the indicated minutes without (–) or with (+) added TPPII inhibitor BUTA.

(E) Structural representations of TPPII highlighting G500D (red spheres) and the active site (purple spheres). Shown are ribbon representations of multimeric spindle and monomer and surface representation of dimer of yellow and green monomers (Schönegge et al., 2012).

Experiments were repeated at least twice for (C) and three times for (D). See also Figure S2.

largely unaffected by genetic deletion of *Tpp2* in mice, even during experimental viral infections (Kawahara et al., 2009). By contrast, other TppII-deficient mouse strains exhibited either embryonic lethality (McKay et al., 2007) or an immunosenescent phenotype characterized by declining thymic output and progressive loss of CD4 and CD8 T cells (Huai et al., 2008). Thus, the physiological role for TPPII in proteolysis, amino acid homeostasis, and metabolism in mammals remains obscure. Furthermore, although humans with loss-of-function mutations in *TPP1* develop a lysosomal storage disease called classical late-infantile neuronal ceroid lipofuscinosis (Tomkinson, 1999), whether *TPP2* mutations cause human disease is unknown.

In the immune system, innate and adaptive cells quickly and coordinately respond to invading pathogens and inflammatory signals. The biosynthetic and bioenergetic demands of the responding leukocytes are extreme because of the sudden requirements for cell growth, trafficking, proliferation, and effector functions. To support this burst of anabolic activity, cellular metabolism radically reorients toward aerobic glycolysis (MacIver et al., 2013; Pearce and Pearce, 2013). Although less efficient in generating ATP, glycolysis generates intermediate metabolites that support biosynthetic pathways for effector functions, including cytokine production (Chang et al., 2013; Shi et al., 2011). It is thus not surprising that metabolic reprogramming is an integral part of leukocyte activation and that a complex regulatory network links nutrient availability with a concerted immune response. Unraveling this complexity is important because of the potential to target metabolic pathways for modulating pathological immune responses. To this end, we have studied patients with a metabolic immunodeficiency caused by *TPP2* mutations.

RESULTS

Human Disease Caused by Loss of TPPII Activity

We identified four patients from two families, affected by combined immunodeficiency, severe autoimmunity, and developmental delay (Figure 1A, Table 1, and Data S1 available online), with biallelic loss-of-function mutations in *TPP2*. Except for P2, who was diagnosed by screening in early infancy, patients presented in early childhood with recurrent bacterial and viral infections of the respiratory tract and middle ear. All three tested patients showed markedly decreased circulating T, B, and natural killer lymphocytes (Figure S1A), including severely