Late-Stage Mitochondrial Dysregulation and Mitophagy Failure

Summary

Tau-positive neurons show a paradoxical up-regulation of mitochondrial oxidative phosphorylation proteins together with proteomic signatures of stalled autophagy and late lysosomal failure. Stratifying by misfolded tau burden (MC1) and pseudotime reveals a compensate-then-collapse trajectory culminating in coordinated mitochondrial–lysosomal decompensation and cytochrome c loss.

Background

Alzheimer's disease (AD) neurons experience progressive proteostatic and organellar stress as tau misfolding and aggregation intensify. Neurons rely on mitochondria for energy and on the autophagy–lysosome system for turnover of damaged proteins and organelles, including mitophagy for removing dysfunctional mitochondria. In this mini-pool proteomic dataset (10 neurons per sample), tau status, misfolded tau load (MC1), and a precomputed pseudotime enable ordering of disease progression. These data afford a stage-aware view of how mitochondrial programs, autophagic flux, and lysosomal function coevolve with tau pathology and how their breakdown could feed forward to exacerbate tau accumulation and neuronal demise.

Results & Discussion

Across tau-positive versus tau-negative neurons, up-regulated proteins are strongly enriched for mitochondrial inner membrane and oxidative phosphorylation terms (e.g., oxidative phosphorylation: FDR $= 3.68 \times 10^{-21}$), with limited enrichment of autophagy/UPS pathways; down-regulated proteins, instead, are enriched for extracellular matrix organization and focal adhesion, rather than the anticipated synaptic or metabolic terms [r1-6]. This unexpected pathway topology suggests that, in tau-positive neurons, mitochondrial machinery is selectively boosted while extracellular matrix programs are suppressed, consistent with a compensatory metabolic response and altered adhesion/structural states. The absence of broad UPS enrichment dovetails with targeted analyses showing no significant UPS protein alterations, focusing attention on autophagy-specific dysfunction rather than global proteostasis failure [r1-11].

Autophagy components show a pattern of initiation under stress with executional failure: SQSTM1 (p62) is massively upregulated (log2FC $= 3.413$, FDR $= 1.76 \times 10^{-8}$) and increases with pseudotime ($\beta = 4.951$, FDR < 0.001), accompanied by upregulation of BECN1 and CTSD but downregulation of ATG12, ULK1, and CTSL, while none of 11 UPS proteins are significantly changed [r1-11]. Autophagy substrate behavior is coherent with impaired flux: of 10 substrates analyzed, all correlate with SQSTM1 and pseudotime ($p < 0.05$), with 8/10 and 5/10 remaining significant after Bonferroni correction ($\alpha = 0.0025$), respectively; 70% show positive correlations (accumulation; e.g., KEAP1) and 30% show negative correlations (depletion; e.g., CAT) with mean |r| of 0.533 (SQSTM1) and 0.467 (pseudotime) [r1-20]. Together, these results position autophagy as the stressed, rate-limiting arm of protein/organelle clearance in tau-positive neurons, with selective substrate accumulation indicating bottlenecks in flux rather than universal pathway shutdown [r1-11, r1-20].

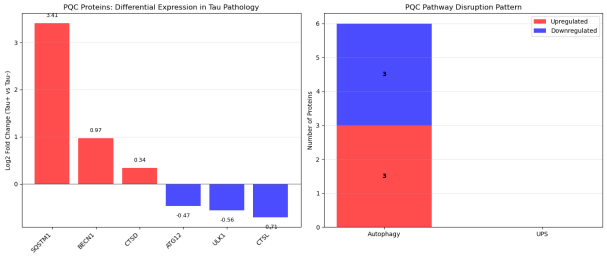

Figure 7: Autophagy is specifically and bidirectionally dysregulated in neurons with tau pathology. (A) Log2 fold change of key autophagy proteins in tau-positive versus tau-negative neurons, showing upregulation of SQSTM1, BECN1, and CTSD, with downregulation of ATG12, ULK1, and CTSL. (B) Summary count of significantly altered proteins demonstrates disruption within the autophagy pathway, while the ubiquitin-proteasome system (UPS) remains unaffected. This expression signature suggests a stalled autophagy process where pathway initiation is attempted but execution is impaired. (Source: [Trajectory r1-11])

Linking mitochondria to autophagy, the coupling between SQSTM1 and VDAC1 (a mitochondrial

outer membrane protein) transforms across disease stages: while the global correlation is negligible ($r = 0.0536$, $p = 0.730$), a running correlation along pseudotime (sliding window $n = 20$) shifts from significantly negative early (mean $r = -0.417$ for pseudotime < 0.33) to positive late (mean $r = 0.478$ for pseudotime > 0.67), with a strong trend ($r = 0.851$, $p = 6.98 \times 10^{-8}$) [r1-31]. This biphasic co-regulation fits a mitigate-then-stall model of mitophagy: early, elevated autophagy (high SQSTM1) associates with reduced mitochondrial markers (VDAC1), consistent with active turnover; later, as flux fails, autophagosomes and mitochondria accumulate together, producing positive co-variation [r1-31]. In parallel, pathway over-representation shows broad upregulation of mitochondrial machinery in tau-positive neurons, plausibly reflecting compensatory mitochondrial biogenesis or accumulation of undegraded mitochondrial proteins as mitophagy stalls [r1-6]. The combined evidence converges on progressive mitophagy failure superimposed on attempted mitochondrial compensation.

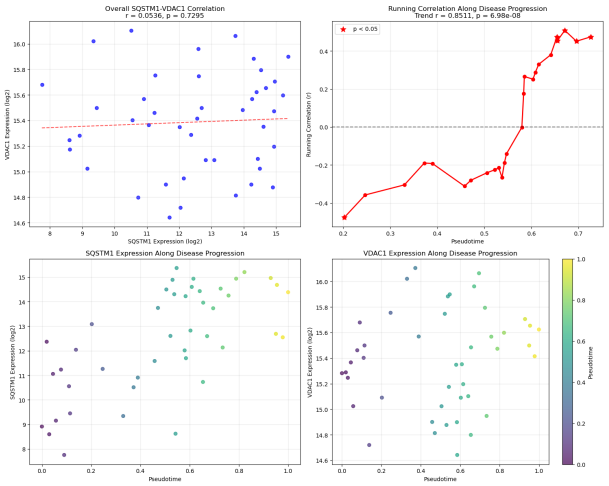

Figure 8: The correlation between the autophagy marker SQSTM1 and mitochondrial protein VDAC1 emerges with disease progression. (A) Overall expression of SQSTM1 and VDAC1 shows no significant correlation across the dataset. (B) A running correlation analysis along a pseudotime trajectory reveals a strong positive correlation developing at late stages. (C, D) Expression levels of (C) SQSTM1 and (D) VDAC1 for each data point plotted against pseudotime. This emergent coupling suggests a coordinated failure of autophagy and mitochondrial homeostasis in late-stage disease. (Source: [Trajectory r1-31])

Late-stage collapse is mapped with two orthogonal readouts tied to misfolded tau burden. First, a V-ATPase Score (a composite of V-ATPase subunit signals) exhibits a segmented, compensate-then-collapse relationship with MC1 in tau-positive samples: a stable early segment (slope = -0.0029, MC1 2.831, $n = 14$) followed by a steep decline (slope = -0.4255, MC1 $>$ 2.831, $n = 8$), with the segmented model outperforming a single linear fit ($F = 4.359$, $p = 0.0286$) and R^2 improving from 0.573 to 0.712 [r1-80]. Second, CYCS (cytochrome c) shows a biphasic pattern: stable/elevated at lower MC1 (MC1 $<$ 2.5: 15.259 $\pm$ 0.167) but sharply reduced at high MC1 (3.0: 14.798 $\pm$ 0.191; $t = 5.474$, $p = 5.097 \times 10^{-5}$, Cohen's $d = -2.58$), with strong negative correlation to MC1 ($r = -0.790$, $p < 0.001$) and positive correlation with pseudotime in tau-positive samples ($r = 0.435$, $p = 0.043$) [r1-25]. CYCS and V-ATPase decline in synchrony (CYCS–V-ATPase $r = 0.696$, $p < 0.001$), consistent with a coordinated mitochondrial–lysosomal decompensation that emerges only after MC1 crosses a critical threshold (≈2.83), locking neurons into a resource-exhausted, pro-degenerative state [r1-25, r1-80]. Integrating these findings suggests a sequence: initial tau misfolding induces compensatory mitochondrial upregulation and autophagy induction; progressive lysosomal insufficiency (reflected by V-ATPase tipping) stalls autophagic flux, driving SQSTM1 and substrate accumulation and coupling to mitochondria (VDAC1); at high MC1, cytochrome c loss and lysosomal failure occur together, consistent with apoptosis-priming and terminal clearance failure, while extracellular matrix program downregulation may further diminish resilience by compromising structural/adhesive support [r1-6, r1-11, r1-20, r1-25, r1-31, r1-80].

Mechanistically, these data argue that tau accumulation is amplified by autophagy-specific failure (SQSTM1 elevation with substrate buildup), not by UPS collapse, and that mitophagy becomes a dominant bottleneck as disease advances, creating a feed-forward loop: damaged mitochondria increase oxidative stress and energy strain, autophagy induction cannot execute due to lysosomal acidification failure (V-ATPase collapse), and cargo—including tau and mitochondrial components—accumulates. The stage-resolved metrics (MC1 thresholding and pseudotime ordering) and dynamic coupling (running correlations) collectively support a late-stage mitochondrial dysregulation with mitophagy failure

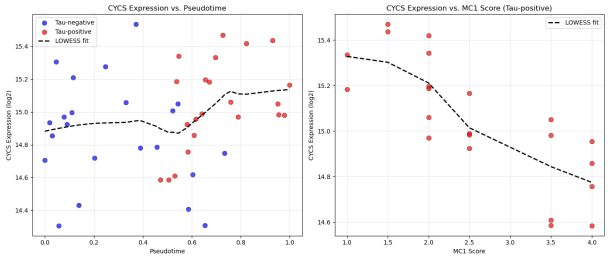

Figure 9: Cytochrome c (CYCS) expression declines with increasing tau pathological burden. (A) Scatter plot of CYCS expression versus pseudotime for tau-negative (blue) and tau-positive (red) neurons. (B) CYCS expression in tau-positive neurons versus the MC1 score for misfolded tau. Dashed lines indicate LOWESS regression fits. The decrease in CYCS expression with higher MC1 scores marks a failure of the mitochondrial compensatory response in late-stage tauopathy. (Source: [Trajectory r1-25])

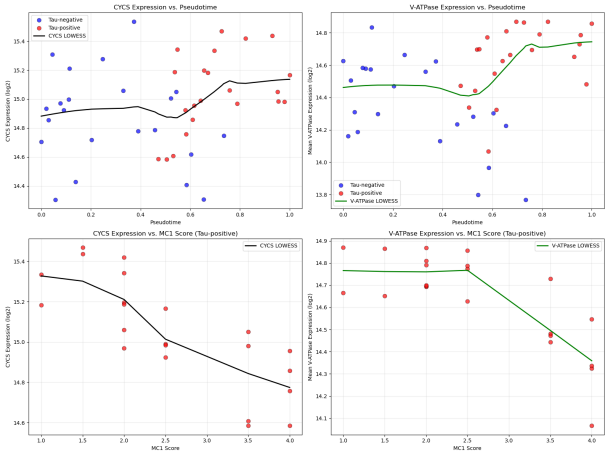

Figure 10: Coordinated decline of mitochondrial and lysosomal proteins marks late-stage tau pathology. Scatter plots show expression of mitochondrial cytochrome c (CYCS, left) and lysosomal V-ATPase (right) versus pseudotime (top) and MC1 tau pathology score in tau-positive neurons (bottom). LOWESS lines depict expression trends for tau-negative (blue) and tau-positive (red) neurons. At high MC1 scores, both CYCS and V-ATPase expression decrease, illustrating a coordinated decompensation of mitochondrial and lysosomal machinery with advancing tau burden. (Source: [Trajectory r1-25])

that is synchronized with lysosomal decompensation, providing a coherent trajectory from compensation to collapse and offering concrete inflection points for therapeutic intervention focused on restoring lysosomal acidification and mitophagy flux before the MC1 $\approx$ 2.83 tipping point [r1-11, r1-20, r1-25, r1-31, r1-80].

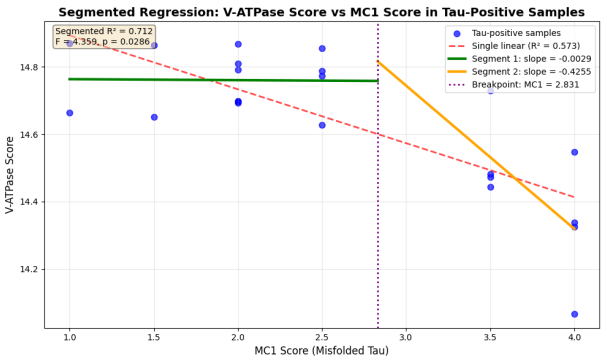

Figure 11: V-ATPase levels decompensate at high levels of misfolded tau pathology. The plot shows a segmented regression of V-ATPase score against the MC1 score for misfolded tau, which remains stable until a breakpoint (MC1 = 2.831) and then sharply declines. This biphasic pattern indicates a late-stage failure of lysosomal acidification machinery, consistent with impaired autophagy. (Source: [Trajectory r1-80])

Trajectory Sources

Trajectory r1-6: The pathway analysis revealed a striking contradiction to the research hypothesis: up-regulated proteins in tau-positive neurons are primarily enriched in mitochondrial oxidative phosphorylation pathways rather than autophagy/UPS pathways, while down-regulated proteins are enriched in extracellular ...

Trajectory r1-11: The PQC system in tau-positive neurons exhibits partial autophagic stress with the key initiator protein SQSTM1 dramatically upregulated (3.4-fold, p<1e-8) while downstream components show mixed dysregulation, but critically no UPS proteins are significantly altered, indicating autophagy-specific dy...

Trajectory r1-20: The analysis provides strong evidence supporting the hypothesis, with 70% (7/10) of established autophagy substrates showing significant positive correlations with both SQSTM1 and pseudotime, indicating progressive substrate accumulation due to failed autophagic clearance.

Trajectory r1-25: CYCS expression shows a dramatic biphasic pattern with stability in low pathological states but a sharp, statistically significant decline at high MC1 levels (Cohen's d = -2.58, p $<$ 0.001), strongly supporting its role as a critical late-stage failure signal that coincides with V-ATPase decline.

Trajectory r1-31: While SQSTM1 and VDAC1

expression show no overall correlation across all 44 samples ($r = 0.0536$, $p = 0.730$), the running correlation analysis reveals a highly significant strengthening of this relationship along the disease progression trajectory (trend $r = 0.851$, $p = 6.98 \times 10^{-8}$), supporting the hypo...

Trajectory r1-80: The V-ATPase Score exhibits a significant biphasic pattern in tau-positive samples, with a compensate-then-collapse response that transitions at MC1 score 2.831.