Inhibition of Transglutaminase as a Potential Therapy in Polyglutamine Repeat Diseases

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Huntington's disease is one model for exploring the roles of the polyQ expansions of huntingtin protein (mhtt) in pathogenesis of disease and pharmacologic intervention. As pointed out in Chapter 48, mhtt leads to formation of insoluble aggregates but can also generate amyloid fibrils as well as oligomeric and protofibrillar structures containing glutamine tracts. These small, soluble species may actually be more toxic and the large insoluble aggregates may in fact be protective (see also (Mastroberardino et al., 2010; Arrasate et al., 2004)). In considering therapy, it must also be kept in mind that while the polyQ fragment may be necessary for toxicity, it may not be sufficient for all of its toxic effects and that more than one pathogenetic mechanism may be involved (see Box 49).

Therapeutic strategies addressing huntingtin gene regulation are discussed in Chapter 48 and histone deacetylase inhibitors in Box 27. The demonstration that polyQ expansions inhibit fast axoplasmic transport through a JNK-activated mechanism undoubtedly opens another fruitful arena of research into potential therapies (Morfini et al., 2009). This essay deals with the background for pharmacologic modification of transglutaminase. The polyQ stretches are substrates for crosslinking transaminations catalyzed by transglutaminases, of which there are four isoforms, the main one in brain being TG2. TG, in a strictly Ca^{2+}-dependent reaction, catalyzes formation of isopeptide bonds between the carboxamide groups of a glutamine residue on one polypeptide with, usually, the epsilon amino group of a lysine residue on another polypeptide (Jeitner et al., 2001). In postmortem HD brain, the total TG activity levels of TG2 protein and TG2-mRNA are increased together with the nuclear aggregates of mhtt. Also, elevations of intracellular Ca^{2+} and the presence of γ-L-glutamyl-L-lysine isopeptides in the mhtt

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aggregates are consistent features in HD brain. Moreover, CSF from HD patients contains elevated quantities of γ -glutamyl isopeptides. These facts are all consistent with upregulation of TG2, increased Ca^{2+} activation of TG2 and increased formation of γ-glutamyl-lysine cross-linkages in HD brain. Elaboration of inflammatory factors and glutamate excitotoxicity with attendant large increases in intracellular Ca^{2+}, which occur in HD brain, may lead to the upregulation of TG2. There is also evidence that mhtt enhances Ca^{2+} release from endoplasmic reticulum, another potential source for activation of TG2 (Jeitner et al., 2001; Jeitner et al., 2009). Thus, increased TG2 activity may result in toxicity in HD through unregulated transglutamination cross-linking of the polyQ with other polypeptides.

To elucidate the role of TG2 in HD, Mastroberardino et al., (2002) generated a transgenic HD mouse model (R6/1) that was also null or heterozygous for TG2. The deletion of TG2 led to significant reductions in the losses of body weight and brain weight, with increases in life span and improvement in motor behavior of the mhtt animals. TG2 ablation also led to large reductions in overall neuronal cell death and reductions in brain isopeptides but also, paradoxically, to an increased number of neuronal intranuclear insoluble aggregates. This suggested that TG2 catalyzed-crosslinking leads to toxicity but not to the formation of the insoluble aggregates and that the non-covalently formed aggregates are probably protective. A direct proof of the toxicity of diffuse polyQ expansions and the protective role of the insoluble aggregates of mhtt was demonstrated in cultured rat striatal neurons by Arrasate et al. (2004).

McConoughey et al. (2010), through a variety of experiments with transgenic mouse, cellular and *Drosophila* models expressing mhtt, showed that (1) TG2 acts in the nucleus to repress transcription factors for key metabolic enzymes; (2) TG2 activity impedes restoration of energy homeostasis after metabolic stresses (such as poisoning with 3-nitrophenol (3-NP),

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deprivation of glucose or oxygen); (3) TG2 inhibition or genetic ablation in mhtt-expressing cells increases mRNA for PGC-1a and *cyto c*, prolongs survival, and normalizes expression of metabolic genes and clusters of other genes; and (4) induces resistance to 3-NP. These experiments are described and referenced in detail (McConoughey et al., 2010). Transcription factors controlling the majority of nuclear-encoded mitochondrial proteins (such as SP-1, Nrf-1 and CREB) have glutamine-rich activator domains and are candidates for TG2-catalyzed cross-linking.

Therefore, investigators have explored the potential of specific TG2 inhibitors. Groups of TG2 inhibitors include siRNA, various small amine-bearing compounds and peptide-based inhibitors. Cystamine, the most widely studied, produces beneficial effects in transgenic mice. However, it also has effects independent of TG2 inhibition, including caspase-3 inhibition and increases in glutathione. However, cystamine is converted to cysteamine, which inhibits transcription of TG. High throughput screening is used to select rationally designed inhibitors that show beneficial effects in model systems (Morfini et al., 2009). One such inhibitor found through peptide screening is termed ZDON (gln-val-pro-leu). It has a higher affinity for TG2 than for TG1 or TG3 and in micromolar concentrations selectively inhibits TG2 in intact striatal neurons in Q7 and Q111 lines *in vitro*, while not inhibiting caspase-3 or raising glutathione levels. ZDON is considered a promising initial tool for developing therapy for HD (McConoughey et al., 2010).

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