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# Targeting HSP90 to Halt Neurodegeneration

**Activation of c-*jun* N-terminal kinase (JNK) signaling is associated with neuronal cell death. As described in this issue of *Chemistry & Biology*, cell-based screening efforts yielded a compound, AEG3482, which interacts with heat shock protein 90 leading to inhibition of JNK and blockade of neuronal apoptosis [1].**

Neurodegenerative diseases, including Parkinson's and Alzheimer's disease, are characterized by the progressive dysfunction and loss of disease-specific classes of neurons. Although these diseases afflict millions, current treatments largely ameliorate symptoms rather than halt disease progression. The majority of neurodegenerative diseases are associated with the formation of protein deposits [2]. Tau-containing neurofibrillary tangles and β-amyloid-containing plaques are characteristic of Alzheimer's, whereas α-synuclein-containing Lewy bodies are found in Parkinson's. In Huntington's and related diseases, polyglutamine-expanded proteins form aggregates. Hsp70 is the chaperone most commonly found in these intracellular inclusions. Protein chaperones may protect against progression of these diseases by promoting the formation of less toxic protein aggregates and/or by targeting toxic, misfolded proteins for degradation through the ubiquitin-proteasome system.

Interruption of neuronal cell death pathways may halt the progression of neurodegenerative diseases. A major event in neuronal apoptosis is activation of the JNK signaling pathway and, indeed, pathological specimens from Alzheimer's disease, as well as samples from mouse models of Alzheimer's and Huntington's disease, reveal activated JNK [3–5]. A well-studied model of JNK-mediated neuronal apoptosis involves deprivation of nerve growth factor (NGF) [5]. In response to NGF withdrawal, JNK is activated through upstream kinases and/or GTPases through a pathway that remains to be completely defined. Activated JNK phosphorylates the transcription factor c-*jun* leading to induction of proapoptotic members of the Bcl-2 family. Some of these Bcl-2 family members are also direct substrates of JNK. Proapoptotic Bcl-2 family members facilitate the release of cytochrome c (as well as other proapoptotic factors) from the intermembrane space of the mitochondria into the cytosol leading to activation of caspases, key proteases involved in cellular destruction.

Because of the important role of JNK in neuronal apoptosis, selective kinase inhibitors that target the ATP binding site of JNK and its activating kinases, including the mixed lineage kinases (MLKs), are being vigorously pursued as potential therapeutic agents [3–5]. In the current study, high throughput screening of a chemical library of 17,000 compounds for small molecules that block NGF withdrawal-induced, JNK-dependent apoptosis of primary sympathetic neurons has led to the identification of an imidazothiadiazole sulfonamide, designated AEG3482, as a potential neuroprotective agent [1]. Indeed Salehi et al. found that AEG3482 inhibited JNK-mediated phosphorylation of c-*jun*, caspase-3 cleavage and activation, and cell death in several cell-based models of JNK-dependent apoptosis, pointing to JNK inhibition as its primary mode of action [1]. However, extended treatment times were required for AEG3482 efficacy; and AEG3482 was unable to block JNK activity in vitro, indicating an indirect means of blocking JNK activation that likely involved transcription and translation of an endogenous JNK inhibitor.

Barker and colleagues hypothesized that AEG3482 may suppress JNK-mediated apoptosis through induction of Hsp70 [1]. Hsp70 has been documented to inhibit JNK by binding JNK and disrupting substrate interactions and/or by stabilizing the phosphatases that would normally inactivate JNK (reviewed by Meriin and Sherman [2]) In accord with this mechanism, AEG3482 treatment increased mRNA and protein levels of Hsp70 [1]. Expression of the Hsp70 gene is controlled by the transcription factor heat shock factor 1 (HSF1). HSF1 is kept in a latent, cytosolic form through its interactions with yet another chaperone, termed Hsp90. In response to cellular stresses, HSF1 is released from Hsp90, translocates to the nucleus, and activates transcription [6]. Further experimentation demonstrated that AEG3482 induced the transcriptional activity of an Hsp70 promoter construct in an HSF1-dependent manner; and multiple lines of evidence point to Hsp70 induction as the means by which AEG3482 blocks apoptosis. The ability of AEG3482-conjugated Sepharose beads to bind purified Hsp90 argues that Hsp90 is its direct target. The aggregate of evidence suggests that AEG3482 protects against neuronal cell death by binding to and inducing a conformational change in Hsp90, leading to HSF1 release and transcriptional activation of Hsp70, which, in turn, blocks JNK activation.

Hsp90 is an abundant, homodimeric molecular chaperone that, in addition to sequestration of HSF1, acts to chaperone steroid hormone receptors and, when in complex with the cochaperone Cdc37, certain protein kinases [7]. The majority of Hsp90 inhibitors, including geldanamycin (GA) and its derivatives, inhibit ATPase activity by competing with nucleotide binding in the N-terminal domain, leading to destabilization and proteasome-mediated degradation of chaperone substrates [8]. Chaperone substrates of Hsp90 include prosurvival and oncogenic kinases like Akt, cyclin-dependent kinases, Raf, and Her2/neu, providing the rationale for exploring Hsp90 inhibitors as anticancer agents; and the GA derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG), is currently in cancer clinical trials [9].

The work of Barker and colleagues is particularly interesting because it suggests that Hsp90, already a target for cancer treatment, may be a viable target for the development of neuroprotective agents. A recent flurry of research activity supports the concept that

mobilization of the heat-shock response protects against neurotoxic insults in a variety of models of neurodegenerative disease [10–15]. For instance, GA treatment protects against the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in a mouse model of Parkinson's disease [14]; and 17-AAG ameliorates neuronal degeneration in a mouse model of the polyglutamine expansion disease spinal and bulbar muscular atrophy (SBMA) [15]. In each of these cases, the classic Hsp90 inhibitors, induce Hsp70 production through activation of HSF1 similar to what was observed for AEG3482. Although JNK activation status was not assessed, it is quite possible that JNK inhibition could contribute to the observed neuroprotective effect.

Interestingly, geldanamycin fails to compete with AEG3482 for Hsp90 binding, suggesting that AEG3482 may not bind through the N-terminal ATPase domain. Furthermore, AEG3482 did not destabilize the prosurvival kinase Akt as geldanamycin does [1, 16], which could certainly be an added benefit in the development of neuroprotective agents. It will be interesting to determine the effect of AEG3482 on other Hsp90/Cdc37 chaperone substrates, including MLK3 [17], which has been implicated in JNK-dependent apoptosis, and the Leucine rich repeat kinase 2 (Lrrk2) [18], which is mutated in a significant fraction of familial Parkinson's disease.

Intriguing data from both the cancer and neurobiology fields suggests that Hsp90 inhibitors may act selectively in pathological cells, sparing normal cells. Despite the ubiquitous, abundant expression of Hsp90, 17-AAG preferentially accumulates in tumor cells as compared with normal cells, consistent with the finding that 17-AAG binds more tightly to the Hsp90 conformer present in activated supermolecular chaperone complexes of tumor cells than to the latent Hsp90 dimer present in normal cells [19–21]. In an $\alpha$-synuclein-induced model of neurotoxicity in fruit flies, GA elevated Hsp70 levels in stressed cells but not in neighboring healthy cells [11]. This may suggest that in the neuropathological state, the chaperone-mediated stress response may be primed for response to Hsp90 inhibitors; and that compounds, such as AEG3482, may be selective neuroprotective agents. It will be important to now assess the efficacy of AEG3482 and related compounds in animal models of neurodegenerative disease. This exciting work of Barker and colleagues, along with other recent findings, suggests that targeting Hsp90 and the stress response may be a promising therapeutic approach for the treatment of diverse neurodegenerative diseases.

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**Selected Reading**

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