Cell-based Assay for NRF2 Activators & NRF2 Activator Scaffolds as Neuroprotective/Antioxidant Agents

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Technology description

NRF2 is a transcription factor and a key regulator of antioxidant defense and detoxification in cells; several big pharma companies are pursuing drugs that activate it as anti-inflammatory competitors to NSAIDs and acetaminophen.
When conditions are normal in cells, Nrf2 is destroyed when a protein called Keap1 binds to a domain in Nrf2 called Neh2. Keap1 in turn binds to other proteins that guide Nrf2 to the proteasome During times of oxidative stress, Keap1 is modified so it cannot bind to Neh2, and as a result Nrf2 persists and sets of transcription of a cassette of anti-oxidant genes.
There are other Nrf2 activation assays available, but none of them directly measure Keap1 interaction with Neh2. The key reagent in our assay is an isolated Neh2-reporter gene contruct, expressed in a cell. If there is signal, Keap1 has not bound to it and sent it to the proteasome; if there is no signal, Keap1 has done its work.

The Cornell team that developed the assay at our affiliate, the Burke Medical Research Institute, used the assay to screen a 2000 compound library. It correctly identified known Nrf2 activators, showed that some putative direct Nrf2 activators actually function via a different mechanism, and identified new Nrf2 activators. The team validated their hits in an astrocyte-neuron coculture model of oxidative stress. The most robust and yet nontoxic Nrf2 activators found—nordihydroguaiaretic acid, fisetin, and gedunin—induced astrocyte-dependent neuroprotection from oxidative stress via an Nrf2-dependent mechanism.

The assay also allows classification of the hits into groups which reflect mechanism of action: (1) immediate activation but gradual stabilization over time; (2) gradual stabilization with a barely detectable (20 min) lag period; (3) gradual stabilization with a short lag period of 40–50 min; (4) stabilization after a prolonged lag period, 1–3 hours; and (5) activation via a switch or receptor, i.e., showing sharp conversion from almost no effect to full activation over a narrow concentration range.

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