Associazione di Biologia Cellulare e del Differenziamento

SIBBM
Società Italiana di Biofisica
e Biologia Molecolare

National Ph.D. Meeting

Salerno, Italy · 24-26 March 2022

Abstracts

https://DOTT2022.azuleon.org

P.17

Analysis of the pathogenetic mechanisms involved in KIF5A-related neurodegenerative disorders

Marta Cozzi[1], B. Tedesco[1,2], R. Cristofani[1], V. Ferrari[1], E. Casarotto[1], M. Chierichetti[3], F. Mina[1], G. Avella[1], M. Piccolella [1], M. Galbiati[1], P. Rusmini[1], V. Crippa[1], C. Gellera[2], S. Magri[2], S. Santangelo[3,4], A. Ratti[3,4], F. Taroni[2], A. Poletti[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy
[2]UOC Genetica Medica – Neurogenetica, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[3]Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milan, Italy
[4]Dipartimento di Neurologia e Stroke Unit – Laboratorio di Neuroscienze, IRCCS Istituto Auxologico Italiano, Milan, Italy

KIF5A is a neuron-specific kinesin heavy chain involved in anterograde axonal transport. Mutations in different KIF5A domains are associated to distinct neurodegenerative diseases (NDs), but the molecular mechanisms underlying such heterogeneity are not known yet. Our aim is to investigate which processes are at the basis of KIF5A-associated NDs by characterizing the effects of different KIF5A mutations, targeting the three domains of the protein (R17Q, R280C, R864X, N999Vfs*39), on KIF5A functions and their impact on neuronal homeostasis.

Upon overexpression of wild-type (WT) or mutant KIF5A in SH-SY5Y cells, altered protein stability/turnover was detected for KIF5A R17Q and N999Vfs*39, which displayed shorter half-life compared to the WT protein upon cycloheximide chase. Moreover, proteasomal blockage by MG132 induced higher protein accumulation for KIF5A R17Q and N999Vfs*39 with respect to WT KIF5A and to the other KIF5A variants, hinting at the ubiquitin-proteasome system as the preferential degradation route for the two mutants. KIF5A R17Q and N999Vfs*39 also mainly partitioned in the NP-40–insoluble fraction upon MG132 treatment, which suggests they might form potentially harmful inclusions upon proteotoxic stress.

Immunofluorescence analysis in NSC-34 cells showed an altered distribution of the overexpressed KIF5A R864X and N999Vfs*39 mutants, that preferentially localized at cell periphery instead of being diffused within the whole motoneuron. In particular, KIF5A N999Vfs*39 mainly formed puncta within cell processes, which further supports the hypothesis of a role for its reduced solubility in pathogenesis. The abnormal distribution displayed by KIF5A R864X and N99Vfs*39 in NSC-34 cells was accompanied by the almost complete lack of colocalization between the two mutants and mitochondria, which are among WT KIF5A cargo.

Altogether, our preliminary data indicate that both unique and shared mechanisms might underlie the pathogenesis of KIF5A-related NDs.