LETTER

Rapamycin induces autophagic flux in neurons

Autophagy is a bulk degradation process initiated when cells engulf cytoplasm (including organelles) within double-membraned vesicles, called autophagosomes, that ultimately fuse to lysosomes, where their contents are degraded. Despite extensive data showing that mammalian target of rapamycin (mTOR) kinase inhibition by rapamycin induces autophagy in organisms from yeast to man, Tsvetkov et al. (1) recently asserted that rapamycin induced autophagy weakly, if at all, in neurons. We feel that this statement needs to be corrected, both for scientific reasons as well as the potential therapeutic uses for this drug in numerous diseases, like Huntington's diseases, where autophagy up-regulation may be beneficial by enhancing degradation of aggregate-prone proteins.

The authors' (1) assertion was based on three pieces of evidence. The first was their figure S1, where they assessed effects of rapamycin on levels of LC3-II, the only known molecule specifically associated with autophagosomes, and not other vesicular structures. LC3-II levels, as a function of actin/tubulin or LC3-positive vesicles, reflect autophagosome numbers in the cell. However, the rate of autophagy cannot be inferred from LC3-II levels or LC3 vesicle numbers, because these can rise because of either enhanced formation or decreased clearance of autophagosomes. Indeed, in some cells, LC3-II levels drop at certain time points after autophagy is induced. Accordingly, no conclusions about rates of autophagy could be drawn from the comparisons made with HeLa cells in their figure S1 (1).

The field has stressed that one needs to assess LC3-II levels in the presence of lysosomal inhibitors, because these block LC3-II/autophagosome degradation and allow one to assess rates of autophagosome formation. As two independent laboratories, we have both shown increases in autophagic flux in primary neurons treated with rapamycin using a range of assays for autophagosome formation (2) as well as assays for autophagosome maturation (2, 3). The second line of evidence presented by Tsvetkov et al. (1) asserted that a previous paper found that rapamycin did not induce autophagy in the mouse brain (4). This study, which showed modest protective effects of a rapamycin analog in a mouse model of Huntington's disease, analyzed brain LC3-II levels without lysosomal inhibitors and found nonsignificant increases. It is interesting to note that others have shown significantly increased LC3 immunoreactivity and LC3-II levels in the brains of rapamycin-treated mice (5). [Fox et al. (4) also measured lysosomal-associated membrane protein 1 (LAMP1) expression; however, we are not aware of data showing that LAMP1 levels reflect autophagic flux.] The third support used by Tsvetkov et al. (1) was one of our papers (3), which was misquoted as showing weak effects of rapamycin in neurons. Actually, we showed robust stimulating effects of rapamycin on autophagic flux, and a conclusion of our study was to stress the importance of assessing autophagy in neurons using LC3-related assays with and without lysosomal inhibitors.

In conclusion, a number of independent studies have shown, using a range of complementary assays, that rapamycin induces autophagy in neurons (2, 3, 5). The data used by Tsvetkov et al. (1) to assert otherwise could not establish this assertion, because the assay that they reported was neither sensitive nor specific.

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