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Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter

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Despite genetic, pharmacological and kinetic evidence validated by a quantitative assay showing that nicotinamide mononucleotide (NMN) is dephosphorylated to nicotinamide riboside before cellular internalization[1], solute carrier family 12 member 8 (Slc12a8), which is widely expressed and annotated as a Na^+/K^+ Cl^- transporter, has been nominated to be an NMN transporter[2]. The analytical methods, transport data and interpretation underlying this assignment are not sound and do not support transport of NMN by Slc12a8.

The article by Grozio et al. reports that Slc12a8, which was previously annotated as an Na^+/K^+ Cl^- transporter, is actually a nicotinamide mononucleotide (NMN) transporter[2]. This would be important if evidence were to support such an identification. However, the authors do not provide such evidence. They used a Hypercarb column to separate acid-extracted metabolites from primary hepatocytes treated with 100 µM NMN in a time course of 0 s, 15 s, 1 min, 5 min, 15 min and 30 min. Problematically, they used one-dimensional liquid chromatography without mass spectrometry to quantify this low-abundance metabolite. The methods cited show an NAD^+ chromatogram illustrating an FK866-depressed peak but no NMN chromatogram; they also acknowledge that the material coming off the column at the NAD^+ retention time contained other analytes when subjected to mass spectrometry[3,4]. The new Fig. 1 in the response by Grozio et al. does not address this problem. It shows that NMN ($m/z = 335$ Da) can be detected in a liver extract but it does not establish the purity of the material in the 22-min high-performance liquid chromatography peak whose area is used for quantification—innumerable other metabolites of different m/z ratios are present in this peak, as the authors have previously acknowledged[3,4]. The new Fig. 2 in the response by Grozio et al. makes matters worse because they did not analyse what an NMN standard looks like in the hepatocyte extract and decided to quantify a peak that has inexplicably drifted from a 22-min retention time to a 19-min retention time.

When one considers that there is approximately 500 times more NAD^+ than NMN in liver samples[5] and that the Grozio et al. do not quantify the 0-s samples, the potential of this liquid chromatography method to produce a signal rather than simply report background is deeply undermined. In fact, Grozio et al. report that incubation of cells with 100 µM NMN—a concentration orders of magnitude higher than cellular NMN[6]—does not produce a time-dependent increase in intracellular NMN (Fig. 1d; the NMN concentration drops from 9–10 nmol mg^{-1} to <6 nmol mg^{-1} in 30 min)[1]. Moreover, the assay for the internalization of ^{3}H-NMN (incubation of ^{3}H-NMN with cells followed by centrifugation through oil) is merely an assay for cell association[2]. Previous work on FK866-resistant NMN incorporation showed quantitative dependence on nicotinamide riboside kinase 1, quantitative extracellular conversion of NMN to nicotinamide riboside and intracellular detection of nicotinamide riboside before formation of intracellular NMN[1]. Moreover, it has been demonstrated that modification of the phosphate in extracellularly applied NMN is required to activate the widely expressed sterile alpha and TIR motif-containing protein 1 (SARM1) to induce an NMN-dependent cell death pathway because NMN itself is not transported[7]. Given the wide expression of Slc12a8, the lack of an analytically sound liquid chromatography–mass spectrometry assay for NMN or an analytically sound transport assay[2] and the extensive earlier work that cannot be explained by NMN transport[1,8–12], it would be prudent to continue to consider that Slc12a8 encodes a salt transporter and not a transporter of NMN.

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References

1. Ratajczak, J. et al. NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. *Nat. Commun.* **7**, 13103 (2016).
2. Grozio, A. et al. Slc12a8 is a nicotinamide mononucleotide transporter. *Nat. Metab.* **1**, 47–57 (2019).
3. Yoshino, J., Mills, K. F., Yoon, M. J. & Imai, S. Nicotinamide mononucleotide, a key NAD^+ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab.* **14**, 528–536 (2011).
4. Yoshino, J. & Imai, S. Accurate measurement of nicotinamide adenine dinucleotide (NAD^+) with high-performance liquid chromatography. *Methods Mol. Biol.* **1077**, 203–215 (2013).
5. Trammell, S. A. et al. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat. Commun.* **7**, 12948 (2016).
6. Trammell, S. A. & Brenner, C. Targeted, LCMS-based metabolomics for quantitative measurement of NAD^+ metabolites. *Comput. Struct. Biotechnol. J.* **4**, e201301012 (2013).
7. Zhao, Z. Y. et al. A cell-permeant mimetic of NMN activates SARM1 to produce cyclic ADP-ribose and induce non-apoptotic cell death. *iScience* **15**, 452–466 (2019).
8. Nikiforov, A., Dölle, C., Niere, M. & Ziegler, M. Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. *J. Biol. Chem.* **286**, 21767–21778 (2011).
9. Grozio, A. et al. CD73 protein as a source of extracellular precursors for sustained NAD^+ biosynthesis in FK866-treated tumor cells. *J. Biol. Chem.* **288**, 25938–25949 (2013).
10. Sociali, G. et al. Antitumor effect of combined NAMPT and CD73 inhibition in an ovarian cancer model. *Oncotarget* **7**, 2968–2984 (2016).
11. Fletcher, R. S. et al. Nicotinamide riboside kinases display redundancy in mediating nicotinamide mononucleotide and nicotinamide riboside metabolism in skeletal muscle cells. *Mol. Metab.* **6**, 819–832 (2017).
12. Vaur, P. et al. Nicotinamide riboside, a form of vitamin B_3, protects against excitotoxicity-induced axonal degeneration. *FASEB J.* **31**, 5440–5452 (2017).

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M.S.S. and C.B. analysed the data. C.B. wrote the manuscript.

Competing interests

C.B. owns stock in and is chief scientific adviser to ChromaDex, Inc. M.S. declares no competing interests.

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