DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

40th CYCLE
Project proposal for a Sapienza PhD scholarship

Main research line

Title: An in vitro assessment of vitamin B12 metabolism defect in neural homeostasis: exploring beyond metabolic abnormalities in the rare VitB12-related disorder Methylmalonic acidemia with homocystinuria, type cblC

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Summary

Vitamin B12 (VitB12), an essential micronutrient, is critical for neural homeostasis, and its deficiency can lead to numerous impairments of nervous system. Several rare conditions associated with defect in uptake of VitB12 or genetic defects in VitB12 metabolism can have detrimental effects at a multi-organ level. However, while VitB12 supplementation generally benefits the human body, achieving rescue at the neural level isn't always attainable. Metabolic alterations due to VitB12 deficiency or genetic errors related to VitB12 metabolism concerning the buildup of toxic products of one-carbon metabolism molecules, such as methylmalonic acid and homocysteine, are well documented, however, little is known about the secondary effects. For instance, these include alterations in S-adenosylmethionine synthesis, which acts as a potent methyl donor and serves as the principal substrate for the methylation of DNA, associated proteins, and RNA.
By utilizing RNA-Seq and microarray datasets obtained from open-source databases, which were generated from heterogeneous experimental models under conditions mimicking VitB12 deficiency, we identified E2F1 and c-MYC, transcription factors, as potential proteins involved in sensing VitB12 alterations. Furthermore, data collected in our laboratory using a differentiated SH-SY5Y human neuroblastoma cell line (exhibiting a neuron-like phenotype) have shown that after oxidative insult, treatment with VitB12 improves cellular homeostasis parameters in accordance with specific trends of E2F1 and c-MYC, indicating a correlation and suggesting potential druggable targets.
Here, we aim at delving deeper into this hypothesis within the framework of the rare VitB12-related disorder known as Methylmalonic acidemia with homocystinuria, type cblC, a condition due to mutations in the MMACHC gene (located at 1p36.3) and is inherited in an autosomal recessive manner. We will establish an in vitro model using a differentiated SH-SY5Y human neuroblastoma cell line specifically engineered with CRISPR/Cas9 to target MMACHC to produce knockout or missense/nonsense mutations (c.80A>G, c.609G>A, c.482G>A, c.394C>T). This model will enable us to investigate the potential role of E2F1 and c-MYC as transcriptional drivers in cellular recovery upon treatment with VitB12. The project will be developed through investigations of immunoprecipitated protein targets (such as E2F1 and c-MYC) to characterize specific post-translational modifications and interactions with other protein partners, and through ChIP sequencing to identify genetic

targets. Additionally, innovative mass spectrometry imaging (MSI, collaboration with Imperial College, London, UK) will be employed for a thorough characterization of metabolic changes. Both our data and literature suggest a shift in the composition of cellular structural lipids in VitB12 deficiency, but little is known regarding cblC. The lipid membrane composition will be investigated using lipidomic analysis and Raman spectroscopy. Also, lipid rafts will be study by labeling and tracking of raft proteins using fluorescence microscope. Computational approaches developed in R software will be utilized to generate correlative images between the spectrometry, spectroscopy and microscopy approaches.

Pertinent Publications of the proponent (last 5 years)

1. Mathew AR, Di Matteo G, La Rosa P, et al. Vitamin B12 Deficiency and the Nervous System: Beyond Metabolic Decompensation-Comparing Biological Models and Gaining New Insights into Molecular and Cellular Mechanisms. Int J Mol Sci. 2024;25(1):590. Published 2024 Jan 2. doi:10.3390/ijms25010590

2. Mathew AR, Cavallucci V, Fidaleo M. Altered vitamin B12 metabolism in the central nervous system is associated with the modification of ribosomal gene expression: new insights from comparative RNA dataset analysis. Funct Integr Genomics. 2023;23(1):45. Published 2023 Jan 23. doi:10.1007/s10142-023-00969-6

3. Fidaleo M, Tacconi S, Sbarigia C, et al. Current Nanocarrier Strategies Improve Vitamin B12 Pharmacokinetics, Ameliorate Patients' Lives, and Reduce Costs. Nanomaterials (Basel). 2021;11(3):743. Published 2021 Mar 16. doi:10.3390/nano11030743

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Mathew AR, Cavallucci V, Fidaleo M. Altered vitamin B12 metabolism in the central nervous system is associated with the modification of ribosomal gene expression: new insights from comparative RNA dataset analysis. Funct Integr Genomics. 2023;23(1):45. Published 2023 Jan 23. doi:10.1007/s10142-023-00969-6

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