Genetically Encoding Unnatural Amino Acids in Neural Stem Cells and Optically Reporting Voltage-Sensitive Domain Changes in Differentiated Neurons.

Journal: Stem Cells

Publication Year: 2011

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PubMed link: 21681861

Funding Grants: Genetic Encoding Novel Amino Acids in Embryonic Stem Cells for Molecular Understanding of Differentiation to Dopamine Neurons

Public Summary:
We describe a lentiviral-based gene delivery method to stably incorporate non-natural amino acids into proteins expressed in neural stem cells. The transduced cells differentiated into neural progenies in the same manner as the wild type cells. By incorporating a fluorescent non-natural amino acid into a voltage-dependent membrane lipid phosphatase, we show that this non-natural amino acid optically reports the conformational change of the voltage-sensitive domain in response to membrane depolarization. The method described here should be generally applicable to other stem cells for non-natural amino acid incorporation, and to the study of many other membrane proteins that are difficult to be studied with conventional methods in vitro.

Scientific Abstract:
Although unnatural amino acids (Uaas) have been genetically encoded in bacterial, fungal and mammalian cells using orthogonal tRNA/aminoacyl-tRNA synthetase pairs, applications of this method to a wider range of specialized cell types, such as stem cells, still face challenges. While relatively straightforward in stem cells, transient expression lacks sufficient temporal resolution to afford reasonable levels of Uaa incorporation and to allow for the study of the longer term differentiation process of stem cells. Moreover, Uaa incorporation may perturb differentiation. Here we describe a lentiviral-based gene delivery method to stably incorporate Uaas into proteins expressed in neural stem cells, specifically HCN-A94 cells. The transduced cells differentiated into neural progenies in the same manner as the wild type cells. By genetically incorporating a fluorescent Uaa into a voltage-dependent membrane lipid phosphatase, we show that this Uaa optically reports the conformational change of the voltage-sensitive domain in response to membrane depolarization. The method described here should be generally applicable to other stem cells and membrane proteins.