

Annual Congress on

# Rare Diseases & Orphan Drugs

October 26-27, 2016 Chicago, USA

## Incorporation of unnatural amino acids restores dystrophin expression in a mouse model of muscular dystrophy

Xia Qing, Jiaqi Lu, Qi Yang, Tianchang Wang, Renyang Xu, Yuyao Tian and Le Tong  
Peking University, China

Premature termination codon (PTC) disease is accounted for 11% of all genetic diseases, including  $\beta$ -thalassemia, cystic fibrosis and Duchenne Muscular Dystrophy (DMD). DMD is a progressive muscular disease affecting 1 out of 3500 male births. This disease is caused by mutations in dystrophin gene. Approximately 10% of mutations causing DMD are non-sense mutations which usually generate a weakly functional truncated dystrophin protein. As a result, myofiber functioning is compromised among patients with DMD. In this study, a genetic code expansion was applied to read-through the non-sense mutation in the dystrophin gene. An evolved orthogonal pair of aminoacyl-tRNA synthetase (aaRS)/tRNA with genetically encoding unnatural amino acid (UAA) selectively enabled the UAA incorporation into the PTC of interested proteins. To further develop a generally applicable UAA system for all three types of non-sense mutations, novel orthogonal tRNAUUA and tRNAUCA were designed to recognize TAA and TGA non-sense mutations. A stable cell line harboring corresponding aaRS/tRNA was built to enhance the UAA incorporation efficiency. During the mRNA translation of dystrophin, the orthogonal tRNA charged with the desired UAA was site-specifically incorporated into the dystrophin protein in response to the non-sense codon TAG. The truncated dystrophin was fully translated by incorporating UAAs into muscle cells manifesting nonsense mutations. UAA plasmids were electroporated and UAAs were injected into the tibialis anterior muscle of mdx mice. Through these procedures, the non-sense mutation was successfully read-through and the full-length dystrophin protein was synthesized. After UAAs were introduced to dystrophin in UAA-DMD transgenic mice, histological characteristics were improved, dystrophin expression in muscle stem cells was restored and muscle functions were recovered. Our results demonstrated that this approach could be applied to treat PTC disease.

xqing@bjmu.edu.cn

## Neurobiology and neurochemistry of Smith-Lemli-Opitz syndrome

Zeljka Korade  
University of Nebraska Medical Center, USA

Smith-Lemli-Opitz syndrome (SLOS) is an inborn error of cholesterol biosynthesis characterized by diminished cholesterol and increased 7-dehydrocholesterol (7DHC) levels. 7DHC is highly reactive, giving rise to biologically active oxysterols. While there is no effective treatment for SLOS, different approaches are in the use to treat various aspects of the disease including antioxidant therapy, inhibition of cholesterol biosynthesis and dietary supplementation of cholesterol. Although there are some improvements cholesterol supplementation does not restore nervous system function. Previous therapeutic approaches concentrated on interfering with the cholesterol biosynthesis pathway and use of statins had limited effect because cholesterol in the nervous system is regulated independently from whole body cholesterol. Dietary cholesterol does not cross the blood-brain-barrier and statins, inhibitors of HmgCoA reductase, in addition to decreasing cholesterol levels, affect cellular signaling and some do not affect brain cholesterol levels because they do not cross blood-brain-barrier. Exogenous cholesterol supplementation does not restore normal cellular function in Dhcr7-ablated cell lines and cholesterol supplementation leads to no significant improvement in SLOS in humans. Thus, it appears that the accumulation of 7DHC and 7DHC-derived oxysterols (rather than cholesterol deficit) may be critical for development of SLOS pathophysiology. Establishing assays for the toxic compounds and understanding their fundamental neurobiology will shed light on SLOS and lead to therapies for this disorder. Novel therapeutic strategies will be presented and discussed.

zeljka.korade@Vanderbilt.Edu