

# **CD44-ERBB RECEPTOR INTERACTION AT THE RAT NEUROMUSCULAR SYNAPSE REVEALED BY FLUORESCENCE RESONANCE ENERGY TRANSFER ANALYSIS BASED ON FLUORESCENCE LIFETIME IMAGING MICROSCOPY**

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CD44 is a multifunctional cell surface glycoprotein which regulates cell-cell and cell-matrix interactions in a variety of tissues. CD44 was implicated in the development of peripheral nerves, functioning as a coreceptor for ErbB class of growth factor receptors. However, it is not known whether CD44-ErbB interaction may occur at the adult peripheral synapses. Here we studied, using Fluorescence Lifetime Imaging Microscopy (FLIM), the proximity between CD44 and ErbB3 at the rat neuromuscular junction (NMJ). This was performed in muscle sections co-immunostained for CD44 and ErbB3, using secondary antibodies coupled to Alexa488 and Alexa647 respectively. Neuromuscular junctions were visualized using Alexa555-bound  $\alpha$ -BT. The occurrence of FRET between Alexa488 (donor) and Alexa647 (acceptor) was judged by measuring an accompanying decrease in the mean donor excited state lifetime in relation to free donor. Two-photon time domain images were acquired using Becker & Hickl FLIM system attached to confocal laser scanning microscope TCS SP2 (Leica). We found that the mean fluorescence lifetime of the donor fluorophore labeling CD44 protein was considerably shorter over the NMJ than in non-synaptic sites. Then we compared normal rat muscle to the muscle affected by denervation upon chronic degeneration of the motor neurons, in the transgenic model of amyotrophic lateral sclerosis (ALS). Importantly, ALS-like neurodegeneration resulted in significant increase in molecular proximity of CD44 and ErbB3 at the NMJ. The specific complex formation between the two proteins was confirmed using immunoprecipitation analysis. Our study provides novel data on the molecular architecture of the neuromuscular synapse in both health and disease.