

Title: Detection of human acid alpha-glucosidase in murine cells and tissues.

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Pompe disease (glycogen storage disease type II) is a rare and debilitating genetic disease characterized by deficiency of lysosomal acid alpha-glucosidase (GAA). This deficiency leads to the aberrant accumulation of glycogen in cardiac and skeletal muscle which causes progressive degeneration of myocytes, impaired muscle function and, ultimately, death. Enzyme replacement therapy (ERT) utilizing recombinant human GAA (rhGAA) currently represents the most promising therapeutic approach. However, the precise cellular and sub-cellular localization of administered enzyme in target tissues remains poorly characterized. To address this need, we have developed and optimized an immunofluorescent staining protocol which employs an anti-rhGAA monoclonal antibody in conjunction with tyramide signal amplification. In combination with laser-scanning confocal microscopy, this protocol allows us to detect internalized rhGAA in cultured murine fibroblasts when administered at concentrations as low as 1uM. The sensitivity obtained is at least 10-fold greater than that observed using standard fluorophore-conjugated secondary antibodies. When applied to frozen tissue sections, this protocol has permitted visualization of administered rhGAA in liver, quadriceps muscle, and heart from GAA knock-out mice. Emission fingerprinting and spectral unmixing have been used to separate actual signal from tissue autofluorescence, further enhancing our ability to detect low levels of enzyme. Work is currently underway to identify organelle- and cell type-specific markers which will allow us to more precisely characterize the cellular and subcellular localization of rhGAA in murine tissues. The ability to evaluate cellular and sub-cellular targeting of ERT therapeutics for Pompe disease will permit more comprehensive evaluation of their efficacy and may provide greater insight as to their mechanism of action.