Localisation and expression of obscurin in failing and non-failing human hearts, using Confocal Fluorescence Microscopy

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Abstract

Striated muscle obscurin belongs to the group of giant proteins that include titin (3600 kDa) and nebulin (700-900 kDa). The obscurin gene (OBSCN) produces multiple alternative-spliced isoforms, which vary in location within the cardiomyocyte. Localisation of obscurin was studied using human left ventricular (LV) donor tissue, stained with antibodies against the N and C-terminal ends of obscurin. Confocal fluorescence microscopy revealed the N-terminal of the two larger isoforms, obscurin A (700 kDa) and obscurin B (800 kDa), to localise to the M-line. The C-terminal antibody targeting obscurin B and the smaller isoform D, localised to either side of the Z-disk in cardiac sarcomeres. Furthermore, Obscurin D showed predominate staining at the cardiac intercalated disc (ID). The location of obscurin at the ID was confirmed via colocalisation with Connexin-43. Although obscurin has been implicated in cardiomyopathies, it is yet to be determined whether the protein can influence or even cause Dilated Cardiomyopathy (DCM). Next generation sequencing has currently identified an obscurin mutation in four DCM patients. To determine any possible downregulation of obscurin at the intercalated disc, DCM patients were compared to age-matched donors, by measuring total fluorescence intensity. One DCM patient showed a 48% decrease in total fluorescence intensity compared to the age-matched donor. This preliminary finding suggests that a truncation of obscurin may occur within a certain population of DCM patients. Further sequencing of new patients is expected to increase the occurrence of this truncation. This will confirm the preliminary finding and point to a possible causative role of obscurin in heart failure.