Doxorubicin-caused cardiomyopathy is due to an inhibition of the fusion step on the autophagy flux

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Doxorubicin (Dox), widely used anti-tumor agent, damages to the various molecules and organelle in the heart by augmenting the oxidative stress, leading to induction of severe cardiomyopathy, while the mechanism of Dox-caused cardiomyopathy remains uncertain. Autophagy is a process that degrades the intracellular accumulated molecules in the lysosome to maintain cellular homeostasis. We have reported that Dox activated the autophagy flux, but caused cell death in a rat myoblast cell line, H9c2 cells used as a model for Dox-induced cardiomyopathy. To further elucidate the mechanism of Dox-caused cardiomyopathy, we first used several cell death inhibitors in the presence of Dox. 3-methyadenine, an autophagy inhibitor, showed a suppress of Dox-induced cell death indicating that autophagy is a primary cause of cell death by Dox. We next evaluated the effect of Dox on the autophagy process and found that it inhibits the fusion step of autophagosome with lysosome to form autolysosome. Since the various membrane trafficking molecules are crucial to regulate the fusion step, we further examined the effect of Dox on the expression of protein X that is potentially control autophagy. As the expression level of protein X was significantly decreased by Dox, we knocked-down the protein X to confirm the role of the protein. Protein X-knockdown cells showed the impairment of autolysosome formation and autophagy flux. These results suggest that Dox-caused the decrease in the expression of protein X resulted in inhibiting the fusion step of autophagosome with lysosome on the autophagy flux.