The pre-autophagosomal structure (pas). autophagy-related (atg) proteins

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Theexact how and from where the autophagosome emerges is unspecified but aresearch has identified a site called pre-autophagosomal structure (PAS). Autophagy-related (ATG) proteins are the main substance involved in the PASleading to autophagosomal production. The discovery of ATG proteins in the1990s greatly advanced the understanding of autophagy and clarified thatautophagy serves important roles in various biological processes. (ShibutaniST1, 2,).

Phagophore, autophagosome and autophagolysosome formation areregulated by at least 30 ATG proteins. (I. M. Aparicio). In recent years, the analysis of knockout models of ATG genes in micerevealed new knowledge about the functions of autophagy in mammalian celldevelopment and differentiation.

Embryos were divided into groups with variousATG genes deleted e. g. ATG5, ATG7, ATG3, etc...and and it was shown that deletionof some ATG genes leads to lethality mid-embryonic development, and the micethat survive postnatal period display some developmental abnormalitiesincluding an atypical lymphocyte differentiation. (Mizushima). In thisstudy, we used cell-lines one with ATG7 deleted gene (KP-4 ATG7-) and ATG5 onewith deleted gene (KP-4-ATG-5) generated using the LentivirusX CRISPR/Cas9System- The lentiviruses encoding the components necessary forCRISPR/Cas9-mediated genome editing for delivery to mammalian cells that aredifficult to transfect. Autophagy-related gene-5 (ATG-5) is one of the keyregulators of autophagic cell death.

It has been widely regarded as aprotective molecular mechanism for tumor cells during the course ofchemotherapy and in recent studies on human gastric cancer, upregulation ofthis gene was associated with chemoresistance.(Ge, Jie et al.) Supportingthese facts are studies revealing that down-regulation of Atg5 expressionsuppresses cell death and vacuole formation induced by IFN-gamma (Jong-Ok Pyo). Inhibition of autophagy by ATG5 and ATG7 gene deletion causes an upregulationof apoptotic markers in response to verapamil, the autophagy inducer used in thisproject. In a research done May 2017, it was discovered that cancer cellstreated with verapamil induce an autophagy flux. Moreover, they found thatinhibition of autophagy in their cell-line (Colon cancer cells) via disruptionof the autophagy genes ATG5 and ATG7 caused an upregulation of apoptoticmarkers (cleaved PARP and cleaved caspase 3) in response to verapamil.

Conclusively, it was found that this response is related to the activity ofLDHA as inhibition reduce both basal and verapamil-induced autophagy, ultimately decreasing cell viability, therefore the potential of usingverapamil with an autophagy inhibitor for cancer treatment. (Orzechowski. A.)