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The role of autophagy in vernal kerato-conjunctivitis

Elena Tarricone [1] - Andrea Leonardi [2] - Antonino Di Stefano [3] - Paola Brun [1]

[1] University of Padova, Department of Molecular Medicine, Padova, Italia - University of Padova, Department of Neuroscience, Padova, Italia [2] – [3] Fondazione Salvatore Maugeri, IRCCS, Istituto Scientifico di Veruno, Veruno (NO), Italia

Autophagy is involved in many biological aspects, including cell survival and death, innate and adaptive immunity and cancer. An involvement of autophagy is also reported in some inflammatory diseases such as asthma. In the present study we explored the role of autophagy in vernal keratoconjunctivitis (VKC), a severe inflammatory disease mainly found in children and adolescents. Autophagy and apoptosis markers (LC3A, LC3B, Beclin-1, cathepsin B, BCL-2, BAX, caspase 3) expression in conjunctival biopsies from 9 active VKC patients and 9 healthy age matched normal subjects were analyzed using immunohistochemistry and qPCR techniques. Conjunctival cells cultures were treated with inflammatory stimuli (IL-1b, histamine, IL-4, TNFa) and analysed by western blotting for autophagy markers expression. LC3B, Catepsin D and B and Beclin-1expression strongly increased in the stroma of VKC whereas the epithelium was consistently negative for all of the molecules studied but positive for Beclin-1 in VKC. qPCR analysis demonstrated a similar mRNAs expression in VKC and normal subjects. In "in vitro" experiments autophagy induction revealed that only LC3B expression was changed in conjunctival fibroblasts by inflammatory stimuli. In particular, both LC3BI, the LC3B free form, and LC3BII, the phosphatidyl-ethanolamine-conjugated form, involved in the autophagosome formation, were decreased in fibroblast cultures at 24h after TNFα stimulation. However, since LC3B-II is normally degraded by lysosomes and the total amount of LC3B-II depends on the balance between its formation and degradation, we analyzed the expression of LC3B-II in the presence and absence of chloroquine, an inhibitor of lysosomal degradation. We found a significant increased amount of LC3BII compared to the control, indicating an over-expression of this protein in stimulated fibroblasts that is quickly damped by its degradation. Since one of the key steps in autophagy is the conversion of LC3B from LC3B-I to LC3B-II, our results suggest that autophagy may be involved in the pathogenesis of VKC.

References

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