

## Niemann-Pick B-lymphocytes show autophagic stress features

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Niemann-Pick disease (NPD) type A and B are lysosomal storage disorders (LSD) due to the lack of acid sphingomyelinase (ASM) activity (Schuchman et al., 2001). The enzyme defect results in a pathological accumulation of sphingomyelin (SM) within lysosomes. In many LSD, an accumulation of undegraded substrates in lysosomes due to deficiency of specific lysosomal enzymes impairs the autophagic process (Settembre et al., 2008), but an imbalance of the of autophagic process in NPB cells has never been shown. The purpose of this study is to examine the autophagic response in NPB B lymphocytes by means of flow cytometry, confocal microscopy and western blot techniques. EBV-transformed B Lymphocytes from patients with Niemann-Pick type B were treated with nocodazole (NZ) and wortmannin (WM), two autophagy inhibitors, and rapamycin (RM), an autophagic inductor. Furthermore we starved cells using a serum-free medium to activate the autophagic process. NPD lymphocytes treated by NZ and RM showed an opposite trend than the expected results for normal cells, in Acridine Orange, LysoTracker Green and CD63 staining, clearly suggesting an impairment of this cellular pathway. Instead, starved cells highlighted a normal behaviour for these markers, indicating a residual ability to enter the process. In conclusion such results suggest the involvement of autophagy and the impairment of lysosomal network before and during NPB cells response to the above-mentioned stimuli. These scenario characterize an imbalance between formation and degradation of autophagic vacuoles (autophagic stress).

### References

- [1] Schuchman E.H., Desnick R.J. (2001) Niemann-Pick disease type A and B: acid-sphingomyelinase deficiencies. In *The metabolic and molecular bases of inherited disease*, 8th edition. Scriver C.R., Beaudet A.L., Sly W.S., Valle D., editors. McGraw-Hill, New York pp. 3589-3610.
- [2] Settembre C., Fraldi A., Jahreiss L., Spampanato C., Venturi C., Medina D., de Pablo R., Tacchetti C., Rubinsztein D.C., Ballabio A. (2008) A block of autophagy in lysosomal storage disorders. *Human Molecular Genetics* 17(1): 119-129.

### Key words

Lysosomal storage disorders (LSD), lymphocytes, autophagy, flow cytometry, confocal microscopy.