Autophago-lysosomal flux following exposure of endothelial cells and fibroblasts to ionizing radiation.

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Objective

Vasculature and connective tissue damage is an important contributor of radiotherapy's side-effects. The aim of this study is to provide insights in the radiobiology of the autophagic response of endothelial cells and fibroblasts.

Materials/Methods

Human vascular endothelial cells (HuVEC) and MRC5 fibroblasts were exposed to 2Gy of ionizing radiation (IR) and an autophagic characterization was performed by confocal microscopy and western blot analysis, at 4 and 8 days post-irradiation using a variety of antibodies for MAP1LC3A and B, LAMP2A, p62 and CathepsinD. Cell proliferation and survival experiments was performed using the AlamarBlue® assay. LC3A and LC3B expression was suppressed using siRNAs.

Results

IR induced accumulation of LC3A+, LC3B+ cytoplasmic vacuoles. The p62/sequstrosome 1 protein was also accumulated suggesting a suppression of the autophago-lysosomal digestive activity. In double immunostaining with lysosomal markers (LAMP2a and CathepsinD) reduction of the co-localization with LC3+ autophagosomes was recorded, suggesting blockage of the "auto-lysosomal flux". Suppression of LC3A/LC3B proteins with siRNAs resulted in radio-sensitization of both cell lines.

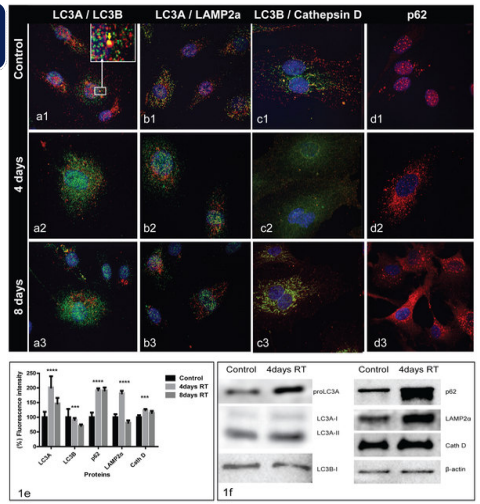

Figure 1. HUVEC confocal immunofluorescent microscopy images LC3A(green)/LC3B(red) (Fig. 1a), LC3A(green)/LAMP2a(red) (Fig. 1b), LC3B(red)/CathepsinD(green) (Fig. 1c) and p62(red) (Fig. 1d) and Western blot analysis(Fig.1e). Co-localization is presented as yellow spots. **Figure 1a**: autophagosomes stained for LC3A (perinuclear localization) and LC3B (cytoplasmic localization). Irradiation-induced accumulation of LC3A and LC3B autophagosomes at both 4 and 8 post-irradiation days (1a2,1a3). **Figures 1b1** and **1c1** show an apparent extent of co-localization of LC3A with LAMP2a and of LC3B with CathepsinD, which was lacking at 4 and 8 post-irradiation days, suggesting autophagy flux suppression (1b2, 1b3, 1c2, 1c3). **Figure 1d** shows poor cytoplasmic and intense nuclear presence of p62 in control cells (1d1); intense p62 cytoplasmic accumulation 4 and 8 days after irradiation (1d2,3). Graphic presentation and quantification of fluorescent intensity of the above immunostaining is shown in **Figure 1e.**

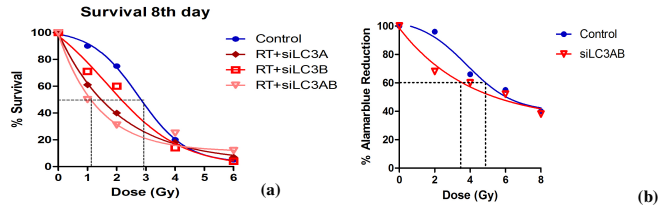

Figure 2. Survival curves at the 8th day for HUVEC (a) and MRC5 (b) . Radiation dose and response curves confirm a shift to the right when a suppression of the LC3A/LC3B expression is achieved before irradiation.

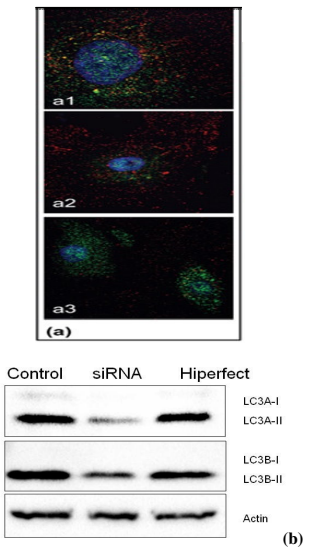

Figure3 . Silencing of LC3A and LC3B. Confocal microscopy for LC3A/LC3B, specific suppression of LC3A (Fig. 3a2) and of LC3B (Fig. 3a3) after incubation with relevant LC3 siRNAs (Fig. 3a). Western blot, confirming specific suppression of LC3A and LC3B in comparison with control and Hiperfect treated cells. (Fig. 3b)

Conclusions

The current data provide evidence that autophago-lysosomal activity in endothelial cells and fibroblasts is suppressed by therapeutic doses of IR and plays an important role in radiation-induced cell damage and eventually the development of tissue toxicities. Future research could focus on autophagy inducers, given that protection of the autophagic flux results in cell protection against radiation damage. These data provide a strong rationale for the development of cytoprotection policies that would have a great impact in the quality of life of patients undergoing curative radiotherapy, or even in the curability of cancer since such policies would facilitate the safe administration of higher radiotherapy doses.

References

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