MACROPHAGE COLONY STIMULATING FACTOR INHIBITS CELL GROWTH OF DIFFUSE LARGE B CELL LYMPHOMA DERIVED CELLS

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INTRODUCTION

Among non-Hodgkin lymphomas, diffuse large B cell lymphomas (DLBCL) are the most heterogeneous subgroup in terms of morphology, clinical presentation and treatment response. Currently, treatment fails for almost half of patients. Mechanisms involved in impaired regulation of apoptosis and cell cycle are involved in DLBCL pathogenesis and progression. Particularly, the expression of antiapoptotic proteins, such as XIAP (X-linked inhibitor of apoptosis protein), survivin, c-IAP1 and Bcl-2 is known to be inhibited by chemotherapeutic agents. However, several groups have been showing macrophage colony stimulating factor (M-CSF) production by DLBCL cells, its receptor expression in tumors, as well as enhanced expression of survivin and Bcl-2 stimulated by M-CSF. Although known by its role in macrophage differentiation, M-CSF is also known to render the immune system permissive for tumors and to act on tumor cells stimulating tumor progression.

Therefore, the present study aims to investigate the effect of M-CSF in DLBCL cells.

METHODS AND RESULTS

DLBCL derived cell lines SUDHL4 and Toledo were treated with 100 ng/ml of M-CSF. Quantification of cells in culture was measured by trypan blue negative cells count. Cell cycle profile was determined by propidium iodide DNA binding and fluorescence detection in at least 10.000 events by flow citometry. Protein expression was evaluated by Western blot. M-CSF exposure in DLCBL cells: (1) reduced cell count in culture, although it did not impair cell cycle profile, (2) decreased protein content of inhibitor of apoptosis proteins (IAPs) cIAP1, cIAP2 and survivin, although it did not alter XIAP and (3) did not alter protein content of Bcl-2 .

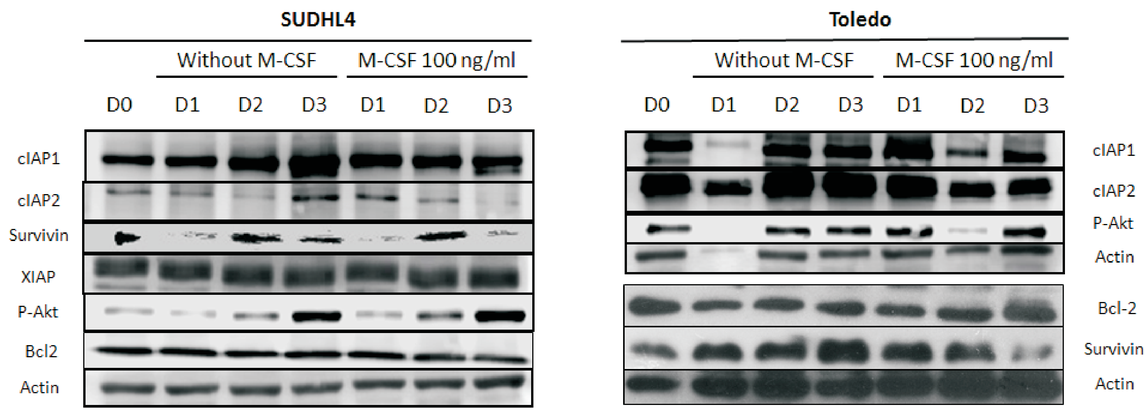

Figure 2: Protein content of cIAP1, cIAP2, survivin, XIAP, P-Akt and Bcl2 upon M-CSF exposure in DLBCL cells. β-actin content was employed as a protein loading control. Legends read: M-CSF adding day (D0), after one (D1), two (D2) or three (D3) days with or without M-CSF 100 ng/ml

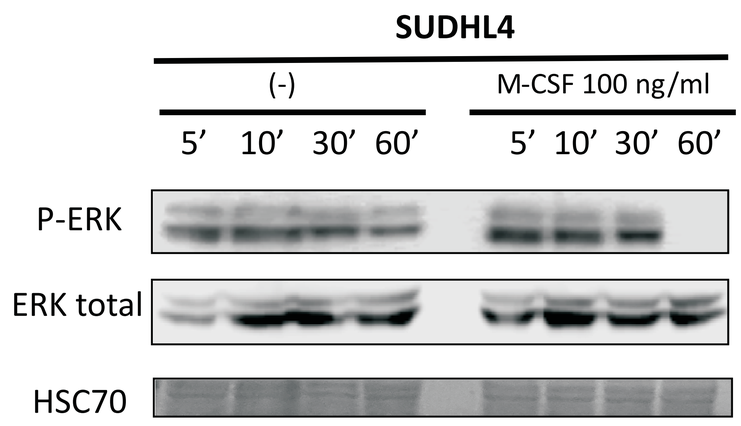

Figure 3: Protein content and phosphorylation (P) of ERK upon M-CSF exposure in SUDHL4 DLBCL cells. HSC70 content was employed as a protein loading control. Legends read: 5 (5’), 10 (10’), 30 (30’) and 60 (60’) minutes with or without (-) M-CSF at 100 ng/ml.

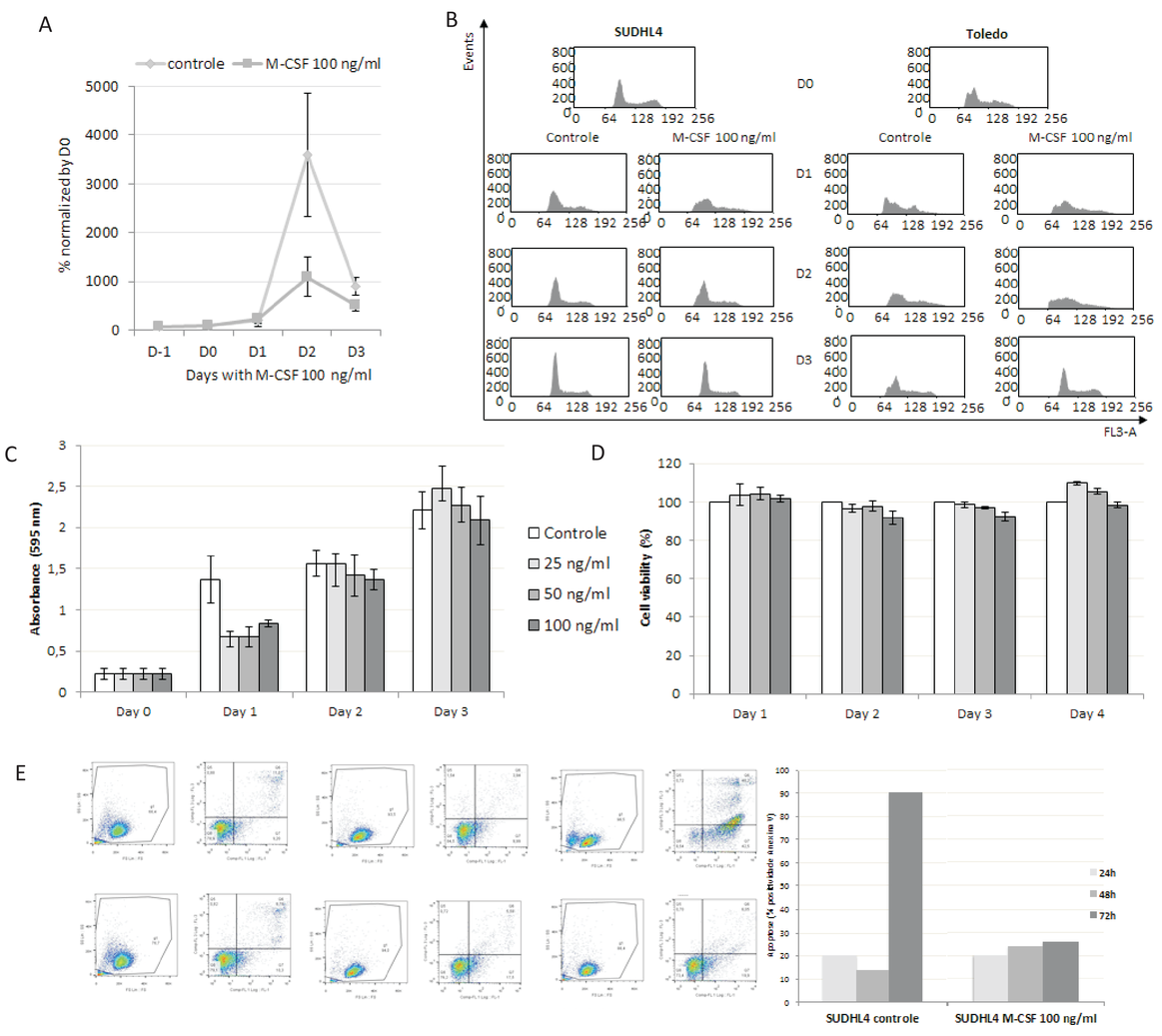

Figure 1: Effect of M-CSF exposure in cell growth of DLBCL cells. A) Cell count of SUDHL4 cells negative for trypan blue at plating day (D-1), at M-CSF adding day (D0) or after one (D1), two (D2) or three (D3) days with or without M-CSF was measured and percentage normalized by day 0 (D0) was calculated. B) Cell cycle profile of SUDHL4 and Toledo cells upon M-CSF exposure for one (D1), two (D2) or three (D3) days. Propidium iodide (PI) fluorescence was detected at the FL3 channel. C) Effect of M-CSF in proliferation of SUDHL4 cells was evaluated by quantification of absorbance after violet crystal cell staining. D) Effect of M-CSF in cell viability of SUDHL4 cells was evaluated by MTT reduction assay. E) Effect of M-CSF in phosphatydilserine exposure of SUDHL4 cells was evaluated by the Annexin V/PI assay. Percentage of Annexin V positive cells were quantified after one (24h), two (48h) or three (72h) days with or without M-CSF. Apoptotic cells Annexin V fluorescence was detected at the FL1 channel and PI fluorescence was detected at the FL3 channel.

CONCLUSION

These data suggest that M-CSF reduces cell growth of DLBCL cells and that this effect is associated with decreased IAPs) cIAP1, cIAP2 and survivin protein content. So far the observed data suggest that M-CSF activates pathways still unknown in these cells and that might have possible implications in therapeutic response.

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Keywords: diffuse large B cell lymphoma, M-CSF, IAPs.

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