LIPOSOMES TARGETING CXCR4 RECEPTORS: ANTI-TUMOR AND ANTI-METASTATIC EFFECTS

S. Lusa[1], G. Salzano[2], C. Ieranò[3], C. D'Alterio[3], M. Buoncervello[5], D. Macchia[5], M. Spada[5], A. Barbieri[4], A. Luciano[4], L. Gabriele[5], C. Arra[4], L. Mayol[2], M. Biondi[2], S. Scala[3], G. De Rosa[2]

[1]Department of Biochemistry and Biophysics, Seconda Università degli Studi di Napoli SUN [2]Department of Pharmacy, Università degli Studi di Napoli Federico II; [3]Oncological Immunology, [4]Animal Facility, [3, 4] Istituto Nazionale per lo Studio e la Cura dei Tumori-IRCCS, Fondazione Pascale; [5]Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità
E-mail: gderosa@unina.it

Purpose: To prepare liposomes conjugated peptides antagonist of CXCR4 receptor to enhance the tumor targeting of these peptides and to promote drug delivery in cancer cells over-expressing CXCR4 receptor.

Methods: The CXCR4 inhibiting peptide (PepR) was synthesized with a sequence of three amino acidis common to the aminoterminal chain of CXCL12, the CXCR4 ligand, and the viral secreted chemokine vMIP-II. Liposomes composed of DPPC/Chol/DSPE-PEG or DPPC/Chol/DSPE-PEG-Mal were prepared. Doxorubicin (DOX) was then encapsulated in liposomes by remote loading. Then, PepR was conjugated with liposomes containing DSPE-PEG-Mal. The size and the zeta potential (ζ) of liposomes, with and without PepR, were determined by photon correlation spectroscopy and Zetasizer Nano Z, respectively. Selected formulations were tested on several cancer cell lines and *in vivo* in an experimental model of lung metastases (C57/BL).

Results: The mean diameter of conjugated and non-conjugated liposomes was found to be between 130 nm and 140 nm, with a narrow size distribution (P.I.≤ 0.1). Moreover, after conjugation of PepR with liposomes, compared with the unconjugated liposomes, showed a significant charge shielding, as demonstrated by the reduction of the ζ from -23 to -6 mV. In the human renal cancer cell (RXF393), PepR, naked or conjugated with liposomes, significantly inhibit cell migration. *In vivo*, mice treated with PepR-conjugated liposomes showed greater reduction of pulmonary metastases, if compared to mice treated with the same dose of naked PepR. When the Lipo-PepR was loaded with DOX, a higher and CXCR4-dependent DOX accumulation was registered in CXCR4 positive cells (A498, HT29), compared with Lipo-DOX; no difference was observed in DOX internalization in CXCR4 negative cells (FB-1). Higher doxorubicin uptake correlated with higher cytotoxicity.

Conclusions: Liposomes conjugation to a CXCR4 antagonist lead to an increase of the *in vitro* and *in vivo* efficacy of these peptides, suggesting an improved antitumor and antimetastatic activity of these new formulations. The DOX-encapsulating Lipo-PepR is a new and powerful tool to target cells with highest migratory and aggressive capabilities (CXCR4 overexpressing cells) and to deliver in these cells cytotoxic drugs.