Iron oxide nanoparticles as drug delivery system for Saporin-mediated breast cancer nanomedicine

Filippo Testa[1], Lucia Salvioni[1], Jessica Bertolini[1], Miriam Colombo[1],Davide Prosperi[1]
E-mail: f.testa@campus.unimib.it
[1] NanoBioLab, department of Biotechnology and Biosciensces, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano

Keywords: nanoparticles, saporin, cancer, nanomedicine, iron oxide, bioconjugation

Abstract: Saporin is one of the most studied plant-derived toxins, belonging to the class of ribosome inactivating proteins (RIPs). Because of its strong cytotoxicity, Saporin has been proposed as a potential new chemotherapeutic drug. However, the proteic nature makes Saporin particularly labile and subject to degradation in the organism, as well as limiting its ability to be internalized by cancer cells.

To overcome these limitations, approaches mainly based on the conjugation of Saporin to antibodies, called immunotoxins (ITs), have been widely proposed and tested. Nevertheless, these ITs showed a good therapeutic potential for haematological cancers but were unable to penetrate and explicate their activity on solid tumours.
Breast cancer cells were chosen as a model for solid tumours and to enhance the penetration and internalization capabilities of Saporin, as well as protecting it from degradation, iron oxide nanoparticles (IONPs) have been used as a vector.

IONPs have been specifically functionalized with a recombinant version of Saporin through the formation of a disulfide bridge. Three batches of IONPs-Sap were produced, with increasing and discrete quantities of conjugated Saporin (1, 2 and 4 molecules of Saporin per nanoparticle), to investigate whether higher numbers of conjugated toxin molecule are correlated with improved antitumoral efficiency.

The effects of IONPs-Sap have been tested on both breast cancer cells (SKBR3) and non-cancer murine fibroblasts (NIH-3T3) in terms of cellular uptake (flow cytometry assay) and viability (MTT assay), protein synthesis inhibition (Click-iT Plus OPP assay) and, lastly, apoptosis and necrosis induction (Annexin V/PI co-staining).

The results altogether indicate high cellular internalization and strong cytotoxicity of IONPs-Sap towards cancer cells, mainly mediated by protein synthesis inhibition and leading to apoptosis induction. On the other hand, while a slight metabolic alteration has been observed, non-cancer cells showed lower cellular uptake of IONPs-Sap when compared to cancer cells and this, hypothetically in combination with poorer endosomal escape capabilities, resulted in a considerably reduced cytotoxicity, with no evidence of both protein synthesis inhibition and apoptosis induction.