Evaluation of HFn-CTX nanoconjugate biodistribution in murine models

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Abstract:
Antibody-based therapy stands as one of the most successful strategies currently employed for treating highly aggressive subsets of cancer. The anticancer effect of antibodies can manifest through direct action, involving specific receptor blockade, or indirect action, engaging immune-mediated cell-killing mechanisms. Unfortunately, the therapeutic efficiency of antibodies (Ab) is still limited due to issues related to their poor pharmacokinetics and penetration of biological barriers. In this context, nanoparticles have emerged as a promising strategy, as they can be functionalized with specific ligands and utilized as vehicles for drug administration. Among these, H-ferritin (HFn), a recombinant form of human apoferritin, has been studied for its biocompatibility, drug-loading capacity, and tumor-targeting capabilities.

We produced endotoxin-free HFn by exploiting an *E. coli* strain containing genetically modified lipopolysaccharide (LPS) that does not elicit an endotoxic response in human cells. Subsequently, we functionalized the surface of the obtained HFn with monoclonal antibody (mAb) Cetuximab. The mAb was conjugated to the HFn nanoparticle using a PEG-based heterobifunctional crosslinker. Via size exclusion liquid chromatography (SEC-FPLC) we isolated the nanoconjugate from the reaction mixture. The resulting HFn-CTX was characterized by SDS-PAGE and Western Blot to qualitatively confirm the successful conjugation.

After testing the biodistribution and cytotoxic activity of the nanoconjugate in 3D tumor models, we proceeded to assess its biodistribution in vivo. The experiment was conducted in female NOD-SCID mice with subcutaneous tumors induced by inoculating luciferase-expressing MDA-MB-231 triple-negative breast cancer cells. After four weeks, mice were injected into the tail vein with the double-labeled nanoconjugate HFn-AlexaFluor647-CTX-AlexaFluor750 at 5 mg kg^{-1}. We monitored the fluorescence emission of the nanoconjugate at 1h, 4h, 24h and 48h post-injection. The fluorescence analysis was performed on the tumor as well as on off-target organs (liver, spleen, heart, lungs, and kidneys) through ex vivo fluorescence imaging with the IVIS system. A fluorescence signal was clearly visible at the tumor site within 1 hour and remained highly detectable after 4 hours. Moreover, we monitored the fluorescence emission in the plasma: our observations showed that the concentration of the nanoconjugate rapidly decreased within an hour after administration. Besides tumors, the major accumulation of nanoparticles was observed in the liver, in accordance with its physiological detoxification role. Confocal images acquired on tumor cryosections revealed a perivascular localization of HFn-CTX at 1h from injection. The next phase will involve assessing the nanoconjugate's potential to induce cell-mediated cytotoxic activity and, consequently, its in vivo antitumor efficacy.