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Cetuximab-Conjugates Nanoparticles for the Treatment of Triple Negative Breast Cancer

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Extended Abstract

Monoclonal antibodies (mAbs) have been developed to improve the treatment of cancer patients. They are able to target certain cancer antigens and possess multiple relevant mechanisms of action (i) modifying the host response to the malignant cells, (ii) delivering cytotoxic moieties, (iii) redirecting T cells towards the cancer to reduce cancer progression inducing an anti-tumour immune response [1, 2].

The research of different formats of antibody fragments, such as single chain variable fragment (scFv), Fab, nanobody, bispecific antibody, bifunctional antibody and half-chain antibodies (HC-mAbs) has been focused on the discovery of valuable alternatives to the nanoconjugation of entire mAbs, which could be able to maintain their functional potential [3].

Many monoclonal antibodies approved for therapeutic purposes or are under clinical investigation. Among them, Cetuximab (CTX): a recombinant human/mouse chimeric monoclonal antibody which binds to the tyrosine-kinase Epidermal Growth Factor Receptor (EGFR). It acts by blocking the dimerization of EGFR thus inhibiting downstream signalling pathways.

CTX-based treatments suffer of several limitations: inadequate pharmacokinetics, short circulation half-life, low tumour penetration and activation of the autoimmune response [4].

In this context, the rise of nanotechnology has offered new opportunities of treatment with mAbs, exploiting new strategies of conjugation to nanoparticles (NPs) or of incorporation within nanocarriers in order to overcome the major limitations of the mAbs-based conventional therapy [5].

Here, we investigated the effect of a CTX-nanoconjugate on triple negative breast cancer (TNBC). TNBC is a particularly aggressive subtype of breast cancer characterized by high heterogeneity and different molecular expression profiles, which make even more difficult the establishment of targeted therapy to control tumour growth [6].

The majority of TNBCs cell lines are characterized by the overexpression of EGFR, which is involved in the control of cell proliferation and survival. EGFR overexpression is associated with poor clinical outcome in patients and has became an emerging therapeutic target for the treatment of TNBC [7].

In the present study, a novel nanosystem consisting of an iron oxide colloidal NPs conjugated with half-chain fragments of CTX (HC-CTX) was developed and tested. The advantage to conjugate HC fragment CTX to colloidal NPs is connected to the presentation of the Fab fragment in the optimal orientation for binding with the receptor in order to improve target recognition.

HC-CTX in vitro efficacy was compared to CTX alone on three different TNBC cell lines, selected according to different EGFR expression, distinctive molecular profiling and various CTX sensitivity. HC-CTX-NPs displayed a high binding specificity to EGFR. The antitumor activity was analysed treating all TNBC cell lines in parallel with increasing

concentration of CTX or HC-CTX-NPs. In general, the viability assay revealed that HC-CTX-NPs displayed a good capability to inhibit proliferation in TNBC cell lines in comparison with CTX alone, affecting proapoptotic molecular mechanism mediated by CTX. The antitumor efficacy of HC-CTX-NPs observed in CTX-resistant TNBC cell lines could be attributed to an alteration of the EGFR receptor recycling which resulted in decrease of the amount of nuclear EGFR due to its sequestration into endolysosomal pathway, as supported by confocal microscopy and Transmission Electron Microscopy.

Besides, we have studied the effect of HC-CTX-NPs on EGFR pathway: CTX-sensitive TNBC cells displayed a high inhibition of EGFR pathway reducing the Y1068 phosphorylation levels, especially at low concentrations.

These results reveal that HC-CTX nanoconjugate amplifies the effect of CTX preserving molecular mechanism involved in CTX activity against TNBC cells. In addition we observed the beneficial effects of HC-CTX nanoconjugate in CTX-resistant TNBC cell lines were attributable to the induction of EGFR degradation.This prevents the fast recycling of EGFR, which results in a decrease of nuclear EGFR levels.

Moreover, the conjugation of HC-CTX to NPs preserves CTX antibody-dependent cell-mediated cytotoxicity (ADCC) response, resulting in enhanced antitumor activity, attributable to multivalent presentation of HC-CTX that amplify NK activation.

In conclusion, this result underlining the potential of this new nanoconjugate in the treatment of TNBC and providing new hope of treatment for this highly aggressive disease.

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