ha eliminato: Effect of cetuximab-5-fluorouracil conjugates on the proliferation of colorectal cancer cells.¶

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Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer worldwide. To date, survival after the diagnosis of metastatic colorectal cancer (mCRC) has improved mostly due to therapies that work towards specific target (targeted therapy), in particular monoclonal antibodies anti–EGFR such as cetuximab (CTX). Primary and secondary resistance due to mutations in the genes codifying for EGFR pathway proteins (RAS, BRAF, PI3K/PTEN) or amplification of other receptors belonging to the ErbB family is the main problem linked to the use of this antibody (Sforza et al., World J Gastroenterol 2016 22:6345). A possible strategy to overcome resistance is represented by the use of antibody-drug conjugates. The group of organic chemistry of the Department of Drug Science and Technology (Turin) has synthetized a series of CTX-5FU conjugates and to increase the number of 5-FU molecules conjugated to CTX, nanodiamonds has been conjugated to CTX (CTX-ND), to synthetize CTX-ND-5FU conjugates.

Materials and methods

The antiproliferative effects of CTX-5FU, nanodiamonds and CTX-ND were tested on a coloncancer cell lines, DiFi, overexpressing EGFR; experiments were performed on wild-type and CTX-resistant cells (DiFi-R) and the antiproliferative effect was evaluated using CellTiterGlo assay (Promega). Modulation of EGFR signalling pathway induced by these conjugates was studied through western blot. Confocal microscopy was used to study the internalization of CTX-nanodiamonds.

Results

CTX-conjugates and nanodiamonds inhibit cell proliferation in a dose-dependent manner. The antiproliferative effect of CTX-5FU or CTX-ND was similar to that obtained treating cells with CTX in DiFi, underling how the conjugation with 5-FU or ND is not able to modify the antibody structure. DiFi-R are more sensitive to CTX-ND than to CTX. These compounds modulate both MAPK pathway and PI3K/AKT/mTOR one. Confocal microscopy demonstrated that CTX-ND enter into cells.

Discussions and conclusion

It is well known that a great problem linked to anti-EGFR therapy in CRC is the primary and secondary resistance. To overcome this problem, we synthetized CTX-conjugates in which CTX was linked to 5-FU (CTX-5FU) or to (CTX-ND), to obtain in future CTX-ND-5FU complexes. Conjugates are able to inhibit cell proliferation in a dose-dependent way and the presence of conjugates don't modify the activity of CTX. In the future we link the two conjugates together, to allow the vehiculation of 5-FU inside the cell to increase this drug concentration in a very selective and targeted way.