EVALUATION OF LR STABILITY BY LC CHIP Q-TOF and quantitative determination of LR peptide cell penetration by LC-MS/MS*

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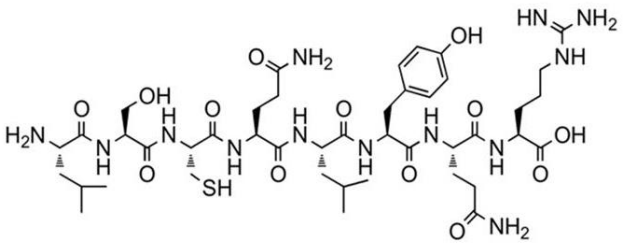
In the present work the degradation profile of an anticancer peptide in different biological matrixes like DMEM (Dulbecco's Modified Eagle Medium) and cell lysates by LC Chip Q-TOF was shown. Subsequently, an LC-MS/MS method for the quantitative analysis of LR in cell lysates was developed and fully validated.



LR peptide

Studio della stabilità di un peptide anticancro in differenti matrici biologiche e sua successiva quantificazione all'interno di cellule di cancro ovarico
Nel presente lavoro è stato studiato il profilo degradativo di un peptide antitumorale in diverse matrici biologiche. Successivamente è stato sviluppato e validato un metodo LC-MS/MS che ha permesso di quantificare la penetrazione del peptide all' interno di cellule di cancro ovarico supportata da un delivery system.

Recently it has been published the synthesis of an octapeptide (LR) which is able to inhibit human thymidylate synthase (hTS) enzyme[1]. Human thymidylate synthase (hTS) is an enzyme of the folate metabolic pathway that plays a key role in DNA synthesis: it catalyzes the reaction converting 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) and it is assisted by N^5,N^{10}-methylenetetrahydrofolate (MTHF) as a cofactor[2]. hTS was found over-expressed in many cancers, including ovarian cancer and, for this reason, has become a drug target for several clinically relevant anti-tumoral drugs[2]. Several inhibitors of hTS are widely used in chemotherapy, therefore, the identification of new compounds able to inhibit hTS is an important topic of pharmaceutical research[3].
Recently, LR oligopeptide was designed to specifically target the protein-protein interface of hTS stabilizing the inactive form of the enzyme[1]. The mechanism of inhibition of hTS involves selective binding to a previously un-described allosteric binding site at the dimer interface of the di-inactive form of the enzyme that involves stabilization of an inactive form of the catalytic protein. In contrast to classical TS inhibitors the lead compound (LR, primary sequence: LSCQLYQR) showed inhibition of cell growth of both cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines inhibiting intracellular TS avoiding induction of hTS over-expression[1].
Although its cytotoxic effect was evident in treated cells, no information concerning the stability in water solution, in culture medium and in cell lysates or the efficiency of internalization within the cells was still available. As the majority of peptides, LR cannot cross the cell membrane and needs a protein-specific delivery system to be transported within the cells; therefore to test the effects of LR on cell growth, it was

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employed a surfactant as delivery system, that did not alter cell growth itself[4,5]. Exploited the fusion, the peptide is released into the cytoplasm of cells.

Recently LC coupled to mass spectrometry detection is widely employed to evaluate low levels of pharmaceutics in different biological matrices, instead of the classical immunological methods. LC-QTOF and LC-MS/MS are powerful tools to elucidate degradation products and to quantify intracellular concentration of pharmaceutics when they are applied to cell lines to test their pharmacological *in vitro* activity[4,5,6,7,8].

The first part of this study has been focused to elucidate the degradation profile of LR peptide in different biological matrixes like DMEM (Dulbecco's Modified Eagle Medium) and cell lysates by LC Chip Q-TOF, identifying its major degradation products. Subsequently, an LC-MS/MS method was developed and validated for the quantitative analysis of LR in cell lysates. An on-line solid phase extraction procedure was employed to reduce the matrix effect and to increase the recovery. Separation of the target peptide and IS was achieved employing C18 column with a gradient elution of mobile phase consisted of 0.1% formic acid and acetonitrile. Sample preparation was optimized in order to precipitate untargeted proteins and to remove interferences from samples. LR peptide was quantified by multiple reaction monitoring mode (MRM). The method was fully validated and it resulted to be selective and linear. The precision, accuracy, recovery and matrix effect were in acceptable range. The fully validated method has been applied to evaluate LR concentration inside cisplatin resistant ovarian cancer cells (C13 cell lines) after different time sets of incubation with the protein transfection system.

In conclusion, using LC-Chip Q-TOF it was possible to identify LR major degradation products; while applying the fully validated LC-MS/MS method it was possible to quantify LR penetration inside ovarian cancer cells, suggesting that the delivery system used represents an effective strategy to deliver the drug inside cells.

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