30P Targeting of BCR-ABL gene expression in K562 cells using cell-penetrating peptide nanocomplexes carrying siRNAs

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Background: Gene silencing by small interfering RNA (siRNA) is a promising therapeutic approach for a wide range of disorders, including cancer. siRNAs against BCR-ABL can be a supportive or alternative measure to traditional chronic myeloid leukemia (CML) tyrosine kinase inhibitors (TKIs) therapies, especially given frequently-noted clinical TKI resistance. The main challenge for such approaches remains the development of effective RNAi systems for intracellular delivery. Cell-penetrating peptides (CPPs) are oligopeptides which have the ability to deliver various cargo molecules. They represent a promising approach of achieving siRNA internalization. Despite the active research mechanisms through which CPPs are internalized remain unclear. It has already been shown that CPPs and their cargoes can be taken up by cells via single or multiple endocytic pathways. Precise siRNA delivery mechanism seems to depend on experimental conditions. In present study we investigated cellular uptake efficiency, internalization mechanism and gene-silencing efficiency of a non-covalent nanocomplex consisting of CPP peptide EB1 and siRNA directed against the BCR-ABL oncogene in the K562 human CML cell line.

Methods: The transfection efficiency of the investigated peptide EB1-BCR-ABL siRNA complexes was measured by flow cytometry analysis. In order to study the complex's internalization mechanisms, transfection was carried out at 4 °C and was analyzed by flow cytometry and confocal microscopy. RNAi modulatory effects were investigated on both mRNA and protein levels of the BCR-ABL using RT-PCR and Western blotting.

Results: We have shown that the transfection efficiency of the investigated complexes was greater than 90%. It was demonstrated that complexes effectively deliver siRNA into K562 cells by endocytic mechanisms. Thereby, transfection of K562 cells by the peptide complexes significantly reduced the levels of BCR-ABL mRNA, with a maximum at 72 h post treatment. Similar decreases in BCR-ABL protein were detected.

Conclusions: Obtained data indicates that CCP-based delivery of siRNAs enables an effective antisense suppression of certain oncogene and represents a promising new class of therapies for CML.

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31P Advantage of co-culture strategy for targeted cancer treatment and in vitro studies

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Background: Breast cancer tissues include carcinoma cells and stromal cells, and intra-tumoral stroma that consists of different types of cells. For this point, cell-cell interaction and communication have a potential role in cancer progression. Mono-cell culture is used for cancer treatment approaches. However, cell-cell interaction and communication can not be evaluated on mono-culture cells. So, co-culture models provide low-cost screening to determine cell proliferation for drug application before moving forward to *in vivo* models. Also, determination of cell morphology in co-culture system is critical to understand advantages.

Methods: In our previous study, novel targeting therapeutic effects of specific micelles including Doxurobicin-loading and unused specific HER-2 peptide sequence (VSSTQDFP) were investigated. The cytotoxicity of micelles is determined on mono-culture as well as the co-culture system of SKBR-3- HER2-(+) and chronic myeloid leukemia cancer cells (K562-HER2-(-)) that constituted an important and first method for drug selectivity studies. During this co-culture study, we observed the differentiation morphology of K562 cells. Following this observation, cell morphology analysis was done on GFP-transfected K562 cells by using the Image J program in terms of size and shape.

Results: In our last study, targeted micelles had more cytotoxicity on SKBR-3 cells to compare co-cultured K562 cells. After this study, the cell differentiation of K562 cells (GFP transfected) was observed after microscopy observation by fluorescence microscopy. After the co-culture of transfected K562 cells and SKBR-3 cells at 24-hours, more than half of K562 cells gained cell adhesion properties. Also, cell morphology in terms of size distribution and shape is different on K562 cells because of microvilli-like structures.

Conclusions: Co-culture studies support novel and well-defined approaches in drug development and determination of targeted drug efficiency. Tumor microenvironment maintains a cell-cell interaction that has a critical role in the initiation and progression of cancer formation. In our future perspective, the cell adhesion molecule, and cytokine signals will be determined to understand cell invasion, migration, and metastasis.

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32P Observational study to compare in vitro sensitivity to therapy in patients-derived organoids (PDOs) with real life outcome: The ChemoSenPDO study

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Background: Despite recent advances in precision oncology, the majority of cases do not present druggable alterations. In such a scenario, treatments must be selected empirically with the only support of general guidelines. Functional tests, able to interrogate the *in vitro* sensitivity to different drugs of a tumor could help to rationalize this decision.

Methods: Onco-PDO© checks the sensitivity of a minimum eight drugs, and their combinations, selected by the attending physician among 100 different options in organoids derived from one given patient. The assay requires a fresh sample with a volume of 1cm^3 obtained by surgery or percutaneous punction. After initial digestion, cells are grown as organoids and exposed to the drugs to establish the percentage of cell death induced by each individual drug. In this study, we aimed to compare the *in-vitro* results with the real outcome of patients.

Results: From August 2019 to April 2021, 67 patients were recruited in four Spanish hospitals obtaining adequate samples in 52. The median age was 57 years (range 4 — 85), 18 were males and 34 females. Tumors included were ovarian cancer (49%), non-small cell lung cancer (24%) prostate cancer (7%), and others 20% (colorectal, melanoma, pancreas, granulosa cells, head-neck, and three pediatric tumors). Organoids could be established in 30 (58%) of the submitted cases. Focusing on the two main tumor types included the success rate for obtaining an OncoPDO© result was 42% in ovarian cancer and 77% in the lung. Drugs in monotherapy that achieved a 50% of cell death in vitro corresponded with clinical benefit in 32% of the cases. This number rose to 50% when assessing combinations.

Conclusions: Studying the *in vitro* drug sensitivity with patient-derived organoids could support a more rational selection of treatments. The observational design of our study, where patients could have received several lines of therapy before the sample for the study was obtained represent a major limitation that should be addressed in the future.

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