

to drawbacks associated with efficiency and viral genome integration. In order to improve reprogramming efficiency and compensate for viral transduction, new chemicals have been explored through iPSC research. The aim of this study was to investigate the proliferative effect of hypericine on human skin fibroblast cells (SF) in-vitro, and to identify the mechanism of action in molecular level.

The proliferation was measured using the Clonogenic and Dimethylthiazol Diphentyltetrazolium Bromide (MTT) assays. Real-time quantitative polymerase chain reaction (qRT-PCR) was performed to detect the mRNA levels of cyclins (D1 and B1) and cell cycle controller genes (p53 and p21).

SF cells were treated with different doses (1 nM–100 µM) of hypericine for 24 h and 48 h. A significant cell proliferation was observed in moderate concentrations (0.1–15 µM; %110–%134), but at high concentrations (25–50 µM) cytotoxic effects emerged in SF cells (IC₅₀ = 23.62 M, $R^2 = 0.915$). qRT-PCR results revealed that the most proliferative dose of hypericine (15 µM) stimulates cyclin D1. The anti-proliferative activity of hypericine was accompanied by inhibition of cyclin B1 mRNA, whereas it induced expression of p53 and p21 genes, and thus apoptosis was observed by DNA laddering at the same dose (50 µM).

Overall results suggested that hypericine can compensate for viral transduction and improve reprogramming efficiency of iPSCs by enforcing them in G1 phase. Hence we report that hypericine can be a good candidate component for cocktails produced to trigger iPSC proliferation.

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Nucleolin overexpression in glioblastoma stem-like cells enables targeted intracellular delivery and improves cytotoxicity

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Glioblastoma (GBM) is the deadliest brain tumor. The mean survival time of GBM patients is approximately 12 months, increasing to 14 months after treatment with temozolomide, which is the *gold standard* chemotherapy. The resistance of GBM to chemotherapy seems to be associated with the blood-brain barrier (BBB) that limits the delivery of chemotherapeutics, and the presence of a population of cells that expresses stem cell-like properties, which are known to be chemo- and radioresistant,⁵ the glioblastoma stem cells (GSCs). The difficulties imposed by these two factors could be reduced by the use of a targeted drug-delivery liposome-based strategy that allows BBB passage and reduces the side effects of chemotherapeutics.

The present study evaluated the ability of the F3 peptide-targeted pH-sensitive lipid-based nanoparticle containing doxorubicin (DXR) to target GSCs and non-GSCs. We evaluated the expression of cell-surface nucleolin by flow cytometry, as well as of stem cell-like markers, in two GBM cell lines. We also determined the ability of GBM cell lines to specifically uptake the F3 peptide-targeted pH-sensitive lipid-based nanoparticles, by flow cytometry, and correlated it with the expression of stem cell-like markers. Moreover, to ascertain the impact of intracellular

delivery of chemotherapeutic drugs into GBM cell lines, cytotoxicity was further assessed by the MTT assay.

Our results showed that the F3 peptide-targeted pH-sensitive lipid-based nanoparticles successfully targeted glioblastoma cells and particularly GSCs. In addition, the results also provided evidence of the nucleolin overexpression-dependency of this strategy, emphasizing the need to adapt the therapeutic strategy to the individual patient.

This study showed that F3-targeted pH-sensitive liposomes may constitute an appropriate strategy to overcome the chemoresistance associated with glioblastoma cells.

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Leukemic cell plasticity as a resistance mechanism towards tyrosine kinase inhibitors

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Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease characterized by the *t(9;22)(q34;q11)* translocation, which encodes the chimeric tyrosine kinase onco-protein, Bcr-Abl. The tyrosine kinase inhibitor (TKI) imatinib is the first-line treatment for patients with CML. Unfortunately drug resistance is one of the main problems observed. While secondary resistance is associated with Bcr-Abl kinase domain mutations, oncogene amplification and mechanisms interfering with intra-cellular drug concentrations; primary resistance mechanisms haven't been elucidated. We generated high dose imatinib-resistant K562 sub-clones (K562-Ir) by clonal selection to study primary resistance mechanisms in vitro. Drug resistance was shown by caspase 3 and annexin V/PI assays. We also showed cellular uptake and function of imatinib with Western blot technics. K562-Ir cells are not only resistant to imatinib but also to 2nd, 3rd generation tyrosine kinase inhibitors. We demonstrated that K562-Ir cells have a highly adherent character, proliferate slowly and are resistant to drug-induced senescence. Microarray analysis revealed that K562-Ir cells differentially express tissue/organ development and differentiation genes at high levels. We showed that K562-Ir cells forms intact tumor spheroids in 3D cell culture conditions which is a marker of tumor initiating potential. Cell surface marker analyses and protein analyses of K562-Ir cell population, points towards an epithelial-mesenchymal plastic cell capable of adopting different morphologies. We hypothesized that imatinib and other tyrosine kinase inhibitors may cause the gain of phenotypic plasticity potential in leukemic cells, by interfering with signalling pathways; which in itself may lead to therapy resistance.