Identification of drugs targeting Leukemic Stem Cell (LSC) signature genes

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Leukemia stem cells (LSCs) play a pivotal role in the process of leukemia initiation, progression, and relapse. For these reasons, LSCs represent a potential and promising pharmacological target for the treatment of leukemia (Zhang et al., 2013). Unfortunately, only few agents have been proven to have a selective effect in the eradication of LSCs. Eppert et al. (2011) defined LSC and hematopoietic stem cell (HSC) gene signatures based on the $CD34^+$ $CD38^-$ subpopulation of 16 primary human acute myeloid leukemia (AML) samples. The aim of the present study is to find and test drugs able to modulate the LSC gene signature and therefore be able to act specifically on the human LSC compartment. For this purpose we used the connectivity map (cmap), which is a collection of gene-expression profiles from five cultured human cells treated with more than 1300 small bioactive molecules. It represents a functional connection between drugs and gene expression (Lamb et al., 2006). We performed cmap analysis using the LSC signature (Eppert et al., 2011) and obtained a list of drugs ranked on the basis of several parameters that express their relative strength in up- or down-regulating the gene signature. We then selected and screened three drugs from this list using two different approaches: vorinostat and trichostatin A, two histone deacetylase inhibitors (HDIs) used for the treatment of cutaneous T cell lymphoma, were chosen since they were the 'top score' drugs; piperlongumine, derived from the fruit of *Piper longum*, was picked as a compound of interest based on its ability to selectively blocks the Nrf2 program in cancer cells, sparing normal cells from toxicity (Raj et al., 2006). The cmap result was first validated by testing the pharmacological activities of vorinostat and trichostatin A on MCF7, the cell line with the highest score in decreasing the expression of the LSCs signature genes. Flow cytometry analysis showed that both drugs were able to induce cytotoxicity, apoptosis, and inhibit cell proliferation. PCR analysis confirmed that the two drugs indeed reduced the expression of the top four significantly down-regulated genes in the cmap analysis (*RBPMS, SETDB1, TRFA3IP2* and *TGIF2*). Experiments were further extended to AML cell lines U937 (monocytic cell line with CALM-AF10 translocation) and MOLM13 (FLT3-ITD positive). We calculated IC_{50} value for all three compounds (vorinostat, trichostatin A and piperlongumine) and all showed the ability to induce apoptosis and to affect cell proliferation in a dose dependent manner. We confirmed by real time qRT-PCR that the two HDIs reduced the expression of *RBPMS, SETDB1, TRFA3IP2* and *TGIF2*. Though all three drugs showed promising results in the selected leukemic cell lines, we further focused only on piperlongumine as it is novel and not well characterized. We investigated its activities on $CD34^+$ sorted subpopulation from 6 primary human AML samples and healthy cord blood. Piperlongumine (0-14 μM) induced apoptosis in a dose-dependent manner and at the highest tested dose completely inhibited the colony formation in all patient samples. Of note, it didn't show any effect on healthy CD34+ cells at all tested concentrations.
Based on these results we can conclude that the cmap is an efficient tool for the identification of drugs active in down-regulating some LSC signature genes and could lead to the identification of potential drugs that could selectively target LSCs and thereby leading to treatment and long-term remission. Piperlongumine is thus a promising pharmacological candidate for further investigations.

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