SMALL MOLECULES DU192, DU283 AND DU325 INDUCE DIFFERENTIATION AND APOPTOSIS OF HUMAN ACUTE PROMYELOCYTIC LEUKEMIA CELLS

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Acute myelogenous leukemia (AML) originates from myeloid stem cells or myeloid blasts halted in an immature state during haematopoiesis. AML represents a group of heterogeneous forms of myeloid malignancies with diverse genetic abnormalities and different stages of myeloid differentiation. The human cell line, HL-60 used in this study belongs to a sub-type of AML, namely acute promyelocytic leukemia (APL). Another pathologic condition of myeloid expansion is the ”emergency” granulo-monocytopoiesis in most of the solid malignancies in which, an army of immature myeloid cells leave the bone marrow, called monocytic and granulocytic myeloid-derived suppressor cells (MDSCs). In contrast to AML, MDSCs are not malignant cells, but promote angiogensesis and immunosuppression leading to the progression of cancer. Both in AML and in solid malignancies the differentiation of immature myeloid cells is an already established therapeutic concept.

Since the differentiation of AML cells is frequently followed by apoptosis or increases the sensitivity to chemotherapy, we have screened a library of small molecules to mature the human prototype cells, HL-60.

In the resazurin assay small molecules DU192, DU283 and DU325 confounded viability of HL-60 cells, half-inhibitory concentration (IC_{50}) values were as follows: 940 nM, 210 nM and 20 nM, respectively. IC_{50} could not be determined for human primary fibroblasts in the applied concentration range (1.6 nM - 5 μM). Using flow cytometry we obtained ERK phosphorylation as an early response to DU325 stimulation followed by the increase of the percentage of the Bcl-xl and pAkt bright cells. The expression of the members of the AP-1 TF complex, a driver of cellular differentiation, c-Fos, JunB, c-Jun and JunD were elevated on a concentration and time dependent manner detected by qRT-PCR. As a proof of cellular differentiation the expression of haematopoietic stem cell markers CD33 and CD34 decreased. Due to maturation the size and granularity of HL-60 cells increased upon treatment. Matured myeloid cell marker CD11b elevated on the cell surface detected by flow cytometry. We confirmed that differentiation of HL-60 cells was accompanied by apoptosis. We could detect $AnnexinV^+/PI^-$ early and $AnnexinV^+/PI^+$ late apoptotic populations after 24h of treatment. Caspase-3 activated gradually by time detected by the percentage of active caspase-3 positive cells by flow cytometry and the digestion of zDEVD – amino-Luciferin. Finally, as a proof of massive cell death, we have shown the appearance of the hypo-diploid apoptotic cells in the sub-G1 population and the leakage of the lactate-dehydrogenase into the supernatant.

We conclude that DU molecules differentiated immature HL-60 cells, which was followed by apoptosis. We propose to further investigate the effects of DU325 on additional human AML cells obtained from clinical samples. On the other hand we plan to systematically investigate the effect of DU325 on the expansion of immature MDSCs in solid malignancies.

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