# Differentiation Induction of Human Leukemia Cells and Normal Hematopoietic Stem Cells by Olive Leaf Extract Components

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## *Abstract*

With the major problems associated with the use of chemotherapy for leukemia treatment, new approaches have been developed during the last three decades. Based on the concept that leukemia cells result from a blockade in the process of maturation, the differentiation-inducing therapy has anticipated as a novel medical treatment aiming to induce leukemia cells to resume the process of differentiation and enter the apoptotic pathways. Even though some success has been made over the past years, problems such side effects during the long term use as well as development of resistance to known differentiation inducers continue to limit this approach. To face these obstacles, transplantation of hematopoietic stem cells (HSCs) and the transfusion of the blood cellular components have been recently come into clinical use and have been shown to be a life-saving procedure in a broad spectrum of hematological diseases. Thus, strategies to expand either hematopoietic progenitors (CD34+ cells) or the selected subpopulations have been an area of active research. This study was launched in an attempt to help the development of new differentiation-inducing agents from olive leaf. Furthermore, the author tried to raise approach for the possible use of phytochemicals from olive leaf as supplement to the *ex-vivo* culture of HSCs in order to direct the stem cell fate decision.

Olive leaves collected from different cultivars of the most abundant Tunisian variety, Chemlali, were screened for their anti-leukemia effects on chronic myelogenous leukemia K562 cells. Ethanol extract of olive leaf from Chemlali Sfax showed the best anti-proliferative effect and was chosen for further study. Results indicated that treatment with Chemlali Olive Leaf Extract (COLE) inhibits the growth rate of K562 cells and caused an arrest of the cell cycle. Further investigations revealed that COLE induces apoptosis and differentiation of K562 cells toward monocyte/macrophage lineage. Microarray analysis was conducted in order to understand the mechanism involved in the described effects. The differentially expressed genes such as *IFI16*, *EGR1*, *NFYA*, *FOXP1*, *CXCL2*, *CXCL3* and *CXCL8* confirmed the commitment of K562 cells to the monocyte/macrophage lineage. Consistently with the increase in apoptotic cell number, treatment with COLE up-regulated some pro-apoptotic genes such as *CASP6*, *CASP8*, *DFFA* and *BID* and down-regulated the apoptosis suppressor gene *BCL2* and the caspase inhibitors. A proposed mechanism for the differentiation and apoptosis-induced effects of COLE, involves a cross-talk between the JNK pathway and the NF-kappaB pathway.

In the second part of our work, olive leaf main phytochemicals were investigated for their potential differentiation-inducing effects on HSCs. Results showed that the main compounds of olive leaf extract, Oleuropein (Olp), Apigenin 7-glucoside (Api7G) and Luteolin 7-glucoside (Lut7G), used alone or in combination, have the potential to induce the differentiation of HSCs into different types of blood cells. The combination of the three compounds enhances the myelomonocytic and lymphoid differentiation while inhibiting the commitment to megakaryocytic and erythrocytic lineages. This effect is likely to be attributed to the combination's major compound Olp. Microarray analysis results showed an up-regulation of

genes related to Wnt signaling pathway (*FZD5, FZD3*), Notch pathway (*NOTCH2, MFNG, APH1B*) and TGF-beta pathway (*TGFBR2, SMAD4*). An up-regulation of genes related to differentiation of lymphocytes B and T was also detected, as well as genes of cell adhesion, motility and membrane trafficking (*CD46, MEF2C, CD58, NAMPT*, etc.). Api7G induced the differentiation of CD34+ cells towards the erythrocytes and megakaryocytes lineages and inhibited the myeloid differentiation, while Lut7G stimulated both the erythroid and the myeloid differentiation. Erythroid differentiation induced by Api7G and Lut7G treatments was confirmed by the increased levels of hemoglobin genes expression (*α-hemoglobin, β-hemoglobin and γ-hemoglobin*) and erythroid transcription factor *GATA1* expression, as well as by the increase in the number of BFU-E and CFU-GEMM in methylcellulose cultures.

Taken together, these findings provide evidence of the differentiation-inducing effects of olive leaf extract and its components on both leukemia cells and normal hematopoietic stem cells. These findings could help designing approaches for the development of diet-derived therapeutic agents against cancer. Moreover, having such effect on hematopoietic stem cells suggests that olive leaf phytochemicals may be used in the development of strategies for stimulating the endogenous stem cells in the adult to promote healing and regenerative medicine. Furthermore, the described properties highlight their potential use in optimizing the *ex vivo* generation of the selected hematopoietic sub-populations for infusion purposes.