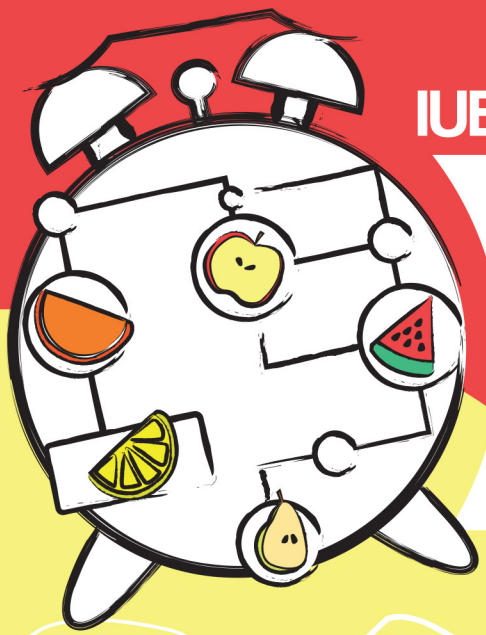

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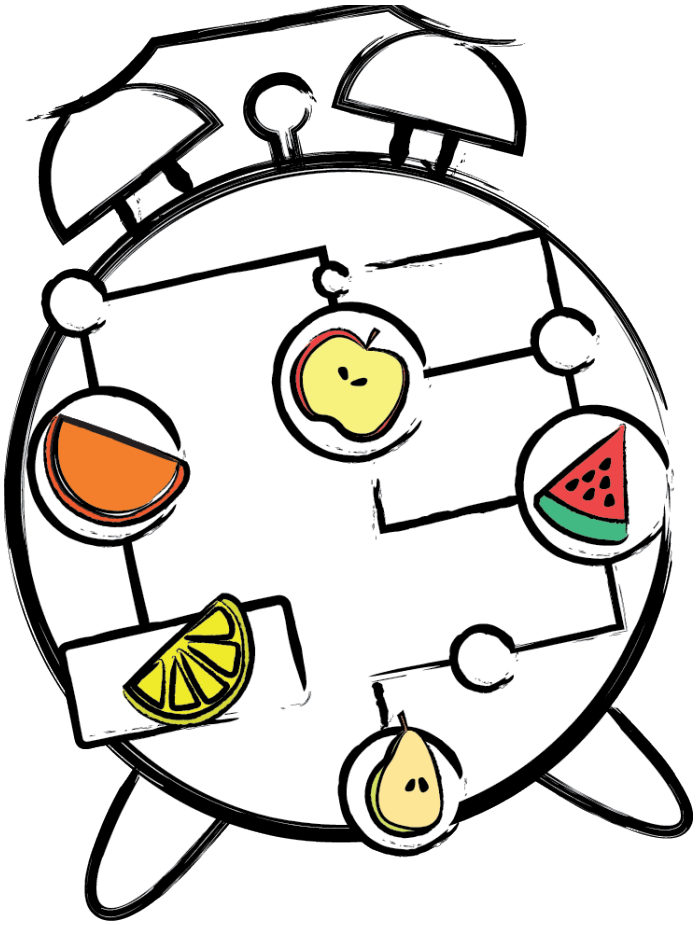

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PHORBOL 12-MYRISTATE 13-ACETATE INDUCES SENESCENCE OF HL-60 LEUKEMIC CELLS

Miloš Mandić[1], Ljubica Vučićević[2], Maja Misirkić-Marjanović[2], Maja Jovanović[3], Ljubica Harhaji-Trajković[2], Vladimir Trajković[1]

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Introduction: Phorbol myristate acetate (PMA) is in clinical investigation for treatment of acute myeloid leukemia due to its differentiating ability. Cell differentiation could be accompanied by senescence, a state of irreversible cell cycle arrest.
Our aim was to investigate the ability of PMA to initiate senescence in HL60 human leukemia cells.
Methods: Cell morphology was analyzed using phase contrast microscopy. Cell cycle arrest was assessed by flow cytometric analysis of propidium iodide stained cells and BrdU colorimetric assay. Activity of senescence-associated beta-galactosidase (SA-βgal) was assessed by cytochemical staining and flow cytometric analysis of fluorescein di-β-D-galactopyranoside (FDG) stained cells. Senescence-associated gene expression of: cell cycle inhibitor p21, interleukin-8 (IL-8), lamin B1 were quantified by RT-PCR, while activation of NF-κB, main regulator of senescence associated secretory phenotype, was examined by immunoblotting.
Results: After the PMA treatment HL60 were enlarged and flattened with cytoplasmic vacuoles resembling morphology of senescent cells. Block in leukemia cell proliferation in G1 phase was accompanied with increase in expression of cell cycle inhibitor p21 in PMA treated cells. Finally, PMA stimulated SA-βgal activity, expression of genes responsible for senescence associated secretory phenotype, NF-κB and IL-8, while downregulating Lamin B1 expression.
Conclusion: Our results suggest that in addition to PMA-induced cellular differentiation, senescence participates in its previously shown cytostatic effect, further supporting its investigation as a potential antileukemic drug.

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