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RETINOBLASTOMA CELLS OVEREXPRESSING THE MRPS18-2 PROTEIN COULD BE DIFFERENTIATED *in vitro*

D.A. SAVIN[1,2], *L.M. KOVALEVSKA*[1], *E.V. KASHUBA*[1]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, Kyiv
[2]Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine

E-mail: *dimasavin133@gmail.com*

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The mitochondrial ribosomal protein MRPS18-2 is involved in cell cycle regulation through its interaction with the retinoblastoma-associated protein, RB. Earlier we have shown that this protein plays an important role in homeostasis of normal and tumor cells, embryogenesis, and in the maintenance of cell stemness. We also found that the MRPS18-2 protein is transactivated by a transcription factor KLF4, one of the Yamanaka factors, inducing cell pluripotency.

Aim. To study the functional consequences of overexpression of the MRPS18-2 and RB (individually and together) in a retinoblastoma cell line (WERI RB 27), concerning putative directed differentiation *in vitro*.

Methods. Cell culturing, cell transfections, cytochemical qualitative reactions for Ca^{2+} ions (Alizarin Red), triglycerides (Oil Red O), and glycosaminoglycans (Alcian blue). Cell morphology was monitored by a phase-contrast microscopy. Direct multipotent differentiation was conducted with cocktails of chemicals for osteogenic, chondrogenic, and adipogenic differentiation.

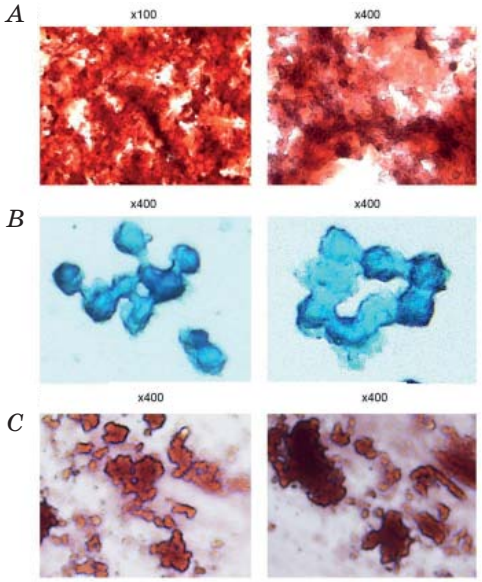

Fig. **A sub-line of WERI-RB-27, overexpressing MRPS18-2, differentiates *in vitro* upon treatment with the specific cocktails of chemicals:**
A — Staining of an osteogenic lineage with Alizarin Red; *B* — Staining of chondrogenic lineage with Alcian Blue; *C* — Staining of triglycerides with Oil Red O

Results and Discussion. Parental retinoblastoma cells are growing in suspension, like bunches of grapes. While subline cells, overexpressing the MRPS18-2 protein, showed slight changes in morphology; the cells form more compact and dense 3D structures. Parental cells WERI RB-27 did not differentiate upon treatment with any of chemical cocktails, cells remained in suspension and did not change morphology. At the same time, cells, overexpressing MRPS18-2 have demonstrated their differentiation ability into osteo-, chondro-, and adipo- lineages. First at all, cells showed changes in morphology and attached to the glass coverslips.

Conclusions. We have shown that overexpression of the MRPS18-2 protein in WERI RB 27 cells leads to changes in cell morphology and to ability of the directed multipotent differentiation *in vitro*. Hence, the MRPS18-2 protein plays a significant role in cancerogenesis and cell stemness.

Key words: MRPS18-2, multipotent differentiation *in vitro*, retinoblastoma, and RB protein.

Authors contribution: DAS and LMK performed experiments with cells and qPCR; LMK and EVK performed immunofluorescent and phase contrast microscopy; DAS performed qualitative reactions; EVK supervised and conceived the study.

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