"DEAD OR ALIVE?" - THE ROLE OF PIBF IN THE REGULATION OF PROLIFERATION AND SURVIVAL MECHANISMS IN B-CLL

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INTRODUCTION: Previously we showed that PIBF - the mediator that prevents embryo rejection and induces active maternal immunotolerance - is constitutively overexpressed by undifferentiated, rapidly proliferating cells and malignant tumors. Further studies revealed that the full-length PIBF is associated with the centrosome and has been identified as a component of the pericentriolar satellite, suggesting its possible role in cell cycle regulation. Recently we found that in certain haematological malignancies (especially in leukemia) the PIBF mRNA and protein expression is qualitatively and quantitatively differs from healthy controls, correlates with the genomic instability and the severity/progression of the disease. These observations raise the possibility that the dysregulated PIBF expression might play an essential role in oncogenesis. Although the role of secreted PIBF in local immunoregulation is well characterized, it is not yet known whether or not PIBF is involved in the regulation of proliferation/survival of different haematological tumors, specially in B-CLL.

METHODS: The expression of intracellular PIBF in B-CLL derived MEC-1 cell line was silenced with (a) synthetic 21-mer duplex siRNA, (b) lentivirus-based shRNA interference technique, then the gene-silencing induced morphological, survival and BCR-related signal transduction changes were characterized. Scrambled siRNA or *Lenilla luciferase*-specific shRNA-transfected MEC1 cells were used as controls. The alterations in cell-proliferation were determined by growth curve analysis and CellTrace CFSE test, the spontaneous and anti-CD95/TRAIL-induced apoptosis was measured with Annexin-V/PI labeling, the mRNS expression of TRAIL-R, OPG, c-$FLIP_L$/c-$FLIP_S$ were analyzed by RT-PCR and the activation of PI3K/Akt, RAF/MEK/ERK and pSTAT3 signaling pathways were studied with Western blotting.

RESULTS: The gene-silencing efficiency of lentiviral shRNAi in MEC-1 cells was significantly higher than that of siRNA constructs (83,2% vs. 6,4-11,9%). Although the PIBF knock-down caused moderate morphological changes, we observed increased spontaneous apoptosis, higher anti-CD95/TRAIL sensitivity and decreased TRAIL-R3/c-$FLIP_L$ expression. Beside this, the BCR activation-related signal transduction pathways that constitutively activated and trigger survival/proliferation signal to B-CLL cells are seriously affected in PIBF-deficient cells: ~~we could detect~~ decreased pAkt-Ser^{473}; pErk1/2 and pSTAT3-Ser^{727}activation was detected that indicate~~ing vigorous~~ a marked down modulation of these pathways.

CONCLUSION: These observations strongly support our hypothesis, that unbalanced PIBF expression is directly involved in tumorigenesis by triggering survival and/or proliferation signals, therefore could be one of the new candidate in the development of rational and targeted tumor therapy.

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formázott: Bal: 2 cm, Jobb: 2 cm, Fenti: 2 cm, Lenti: 2 cm, Élőfej távolsága a lap szélétől: 1,25 cm, Élőláb távolsága a lap szélétől: 1,25 cm

formázott: Betűtípus: Nem Félkövér