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MNT-REL novel interaction: evidences of a role of MNT on NF?B pathway

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Introduction. MYC-MXD superfamily includes proteins that play an important role in cancer. One of the mechanisms that regulate MYC activities is the antagonism played by MNT and other MXD proteins. Both MYC and MXD proteins are basic helix-loop-helix leucine zipper (bHLHLZ) transcription factors that heterodimerize with MAX, bind to E-boxes within regulatory regions of target genes, and generally activate (MYC) or repress (MXD) their transcription. However, some MAX-deficient cell lines and tumors with MAX mutations have been described, pointing out the existence of unknown MAX-independent functions of MYC and MXD proteins. Our preliminary results in UR61 cells (derived from rat pheochromocytoma and deficient in MAX) based on proteomic analysis suggest a possible interaction between MNT and c-REL (REL) that is MAX-independent. REL belongs to the REL/NF?B family that takes part in several biological processes, such as immunity, inflammation, differentiation, cell growth, tumorigenesis and apoptosis.

Objectives. First, to confirm the interaction between MNT and REL found in the proteomic analysis in different human and murine cell lines. Second, to explore the effect of the knockdown of MNT on the NF?B signaling pathway. Finally, to study whether MNT and REL knockdown affects cell proliferation.

Methods. The cell lines used in this work were MAX-deficient UR61 (rat pheochromocytoma), together with C6 (rat brain glioma), Neuro-2a (mouse brain neuroblastoma), LoVo (human colorectal adenocarcinoma) and CEM (human acute lymphoblastic leukemia, T lymphoblast). Co-immunoprecipitation and proximity ligation assays were used to confirm MNT and REL interaction. Immunofluorescence was carried out for studying subcellular localization of NF?B proteins. Luciferase activity of reporters containing the I?B? promoter's sequence or several ?B binding sites was tested in UR61, C6 and LoVo cells for exploring the effect of the MNT knockdown on the NF?B pathway. Lastly, to determine the proliferation rate of cells after MNT and/or REL silencing, clonogenic assays were achieved by puromycin selection for 7-15 days, crystal violet staining of the colonies, dye solubilisation and determination of the absorbance.

Results. The interaction of MNT with REL was found in UR61 and other murine (C6, Neuro-2a) and human (CEM and LoVo) cell lines by co-immunoprecipitation assays, as well as the interaction with p50, another member of the REL/NF?B family, which could be in the complex. This interaction was not found in other cell lines tested. Then, the possible role of MNT in NF?B's pathway was analysed by performing its knockdown with a short-hairpin construct in UR61, C6 and LoVo cells. Reduced levels of MNT caused an increase in the activity of luciferase reporters carrying NF?B response elements and the translocation of REL into the nucleus, as assessed by immunofluorescence assays. However, p65 did not change its subcellular distribution, suggesting that the role of MNT in NF?B pathway is specific for its interaction with REL. Furthermore, we showed that MNT and REL knockdown, both separately and together, reduced cell proliferation of UR61 and C6 cells, but their effects were not additive, pointing out to a possible role of MNT and REL in the same pro-survival pathway.

Conclusions. In summary, in this study MNT-REL interaction was confirmed in LoVo, CEM, UR61, C6 and Neuro-2a cell lines, but not in other human and murine cell lines tested. p50 co-immunoprecipitated with MNT in LoVo and CEM cells, so it may be in the complex. Then, our data suggest a role of MNT in the NF?B pathway as its knockdown caused a translocation of REL into the nucleus in LoVo cells, but not of p65, together with an increase in NFkB's activity. Finally, MNT and REL knockdown was observed to reduce cell proliferation of UR61 and C6 cells.