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RUK/CIN85-OVEREXPRESSING 4T1 BREAST CANCER CELLS WITH AMOEBOID PHENOTYPE ARE SENSITIVE TO THE ANTI-METASTATIC ACTIVITY OF 1,25(OH)₂D₃

Background/Aim. Previous studies demonstrated high levels of adaptor protein Ruk/CIN85 expression in human tumors of different tissue origins [1]. Up-regulation of Ruk/CIN85 in mouse 4T1 breast cancer cells led to acquisition of aggressive amoeboid-moving phenotype dependent on RhoA/ROCK signaling. Several reports showed that 1,25(OH)₂D₃ can modulate RhoA/ROCK signaling [2]. The aim of the study was to investigate the regulatory interrelation between Ruk/CIN85 and vitamin D3 receptor (Vdr) expression levels, the effect of vitamin D3 on metastatic activity of 4T1 RukUp cells *in vivo*. **Methods.** 4T1 cells overexpressing Ruk/CIN85 (RukUp) and control cells were used. Cell viability and protein content was analysed using the MTT assay and Western-blotting, respectively. For studies *in vivo*, a model of experimental metastasis was used. **Results.** 1,25(OH)₂D₃ (1–100 nM) reduced viability of 4T1 RukUp cells. While total VDR levels remained unchanged, nuclear VDR content was approximately threefold higher compared to control, indicating sensitivity of 4T1 RukUp cells to VDR signaling. Intravenous injection of 4T1 RukUp cells into Balb/c mice produced metastases in the lungs, heart, and liver. Histological analysis revealed that metastases localized mainly to peripheral perivascular zones formed protective cuffs around blood vessels and frequently extended diffusely into the surrounding parenchyma. In contrast, intraperitoneal administration of 1,25(OH)₂D₃ to mice injected with 4T1 RukUp cells resulted in only rare cases (1/6) of perivascular metastases, which displayed low mitotic activity and high levels of apoptosis. In the lungs of five mice, pronounced perivascular lymphocytic infiltration was observed. **Conclusions.** We demonstrated the therapeutic potential of 1,25(OH)₂D₃ to suppress metastasis of 4T1 RukUp cells by reprogramming them toward a less invasive phenotype. This effect appears to be mediated through interference with RhoA/ROCK-driven amoeboid motility thereby reducing the ability of tumor cells to transmigrate through tissue barriers. **Grants/Fundings.** The present study was supported by Grants from NRFU N2020.02/0195 and N2025.07/0367.

Keywords: carcinogenesis, 4T1cells, Ruk/CIN85, 1,25(OH)₂D₃.

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