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Functional analysis of RAB27 in melanoma cell lines: small extracellular vesicle secretion, migration, invasion and cell signalling

Abstract

Melanoma is the deadliest of skin cancers, and the number of patients diagnosed with it increases every year. When detected early, it is almost completely curable, but advanced stages of this disease are usually associated with low survival rates. Current targeted therapy with BRAF and MEK inhibitors, as well as immunotherapies targeting CTLA-4 and PD-1, show limited efficacy. Most patients have innate or acquired resistance to treatment, contributing to melanoma's high mortality rate. Additionally, melanoma has the highest mutation rate among all cancers, partially due to the significant role of ultraviolet radiation in its formation. Therefore, ongoing research focuses on identifying new molecular therapeutic targets to support its treatment.

The main goal of this study was to understand the role of RAB27 in melanoma cells. This protein exists as two isoforms, RAB27A and RAB27B, and is involved in numerous physiological and pathological processes. Various studies indicate an oncogenic character of RAB27 in multiple types of cancer. This effect is primarily attributed to promoting the secretion of small extracellular vesicles by cancer cells. The released vesicles act as carriers of oncogenic nucleic acids and proteins, enabling communication between tumor cells, the tumor microenvironment, and distant tissues. Vesicular transport contributes to the formation of premetastatic niche, malignant transformation of normal cells, or immunosuppression. However, independent of vesicular release, RAB27 also affects the secretion of soluble factors (e.g. cytokines, metalloproteinases) that promote tumor progression and modulate cellular functions such as proliferation or migration.

In this study, I used three melanoma cell lines: SkMel28, DMBC12, and A375 with CRISPR/Cas9-mediated silenced expression of RAB27A, and the A375 cell line with silenced expression of both RAB27A and RAB27B. The study was conducted in three steps. After generating the knockout cell lines, the small extracellular vesicles released by KO cells were characterized in comparison to those from wild-type cells. It was shown that the number and size of vesicles were not altered by RAB27A or RAB27A/B silencing. The total protein concentration was comparable in vesicles from WT and RAB27A KO SkMel28 and DMBC12 cell lines but was higher in vesicles from A375 knockout cells, compared to WT. Significant differences in the levels of proteins characteristic of small extracellular vesicles - tetraspanins CD63 and CD81, and ESCRT proteins TSG101 and Alix - were observed in all studied cell lines. However it is noteworthy that these differences were cell line-dependent, and there was no overall correlation between RAB27A levels and vesicular proteins.

Further studies focused on the functioning of melanoma cells with RAB27A or RAB27A/B knockout. Cell proliferation was assessed by measuring the DNA growth, the wound healing assay was used as a model of two-dimensional migration, and cell migration through the extracellular matrix layer acted as a model of invasion. Among all studied cell lines, only SkMel28 RAB27A KO cells showed inhibited proliferation. In contrast, both SkMel28 RAB27A KO and DMBC12 RAB27A KO cells exhibited limited migration and invasion. A decrease in the level of N-cadherin, a mesenchymal marker, was also observed, further suggesting that RAB27A regulates the invasiveness of these melanoma cell lines. In contrast, the motility of A375 RAB27A KO cells remained unchanged, while the double knockout cells showed inhibited migration. Since the A375 line is characterized as the most invasive, this leads to the assumption that RAB27A affects only the functioning of less aggressive cells.

In the final stage of this study, the levels of proteins involved in oncogenesis and/or tumor progression were assessed. By examining the proteomic profile, I identified numerous proteins with increased or decreased expression due to the silencing of RAB27A or RAB27A/B. Among these proteins were HER family receptors, whose levels were significantly altered. To further explore the interaction between RAB27 and HER receptors I measured mRNA and protein levels and surface expression of HER2, HER3, and EGFR. The results showed that silencing RAB27A significantly reduced HER3 levels in all studied melanoma cell lines. In contrast, the fluctuations in HER2 and EGFR levels varied between cell lines. Due to the direct involvement of HER family receptors in RAS/RAF/MEK/ERK and PI3K-AKT signaling pathways, I analyzed the activation of AKT and ERK1/2 proteins. It was shown that the phosphorylation of ERK1/2 and AKT proteins was decreased in DMBC12 RAB27A KO cells, while it remained unchanged in SkMel28 RAB27A KO and A375 RAB27A KO cells. Reduced AKT phosphorylation was also observed in A375 RAB27A/B double knockout cells.

In conclusion, the results of this study provide a better understanding of the functions performed by RAB27A in melanoma cells. It was shown that RAB27A does not directly affect the number of released small extracellular vesicles but significantly shapes some of their protein content. Moreover, RAB27A regulates the proliferation, migration, and invasion of melanoma cells in a cell line-dependent manner. In addition, an interaction between RAB27A and HER family receptors affecting the activation of signaling proteins was discovered. The results presented here suggest that RAB27A plays a role in melanoma progression, but the degree of its involvement is strictly cell line-dependent. Therefore, it seems unlikely that the RAB27A protein is a universal therapeutic target in melanoma.