Carcinoma of the thyroid gland is one of the most frequent malignancies of the endocrine system and its incidence has been steadily increased in many regions of the world. Among the most frequent genetic alterations occurring in thyroid transformation, oncogenic mutations of RAS family genes have been identified in all types of thyroid malignancies, and are considered to be both an early and late event in thyroid tumorigenesis. Indeed, the expression of the oncogenic RasR12, induced by tamoxifen in FRTL5 rat thyroid cells, caused complete malignant transformation towards an undifferentiated phenotype, confirming that Ras could contribute to the partial or complete loss of differentiation, characteristic of the more aggressive thyroid cancers (1). Oncogenic Ras is able to induce aberrant expression of mRNAs during the transformation of thyroid epithelial cells (2). Among the up-regulated mRNAs there is miR-29b, a mRNA that we showed to directly target PATZ1, a protein belonging to the POU and Zinc finger family of transcriptional factors. Recently, we found that PATZ1 is downregulated in thyroid cancer and acts as a tumor suppressor in human thyroid cancer cells mainly by inhibiting cell migration and partially reverting the Epithelial-Mesenchymal Transition (3). Subsequently, we found that it is specifically downregulated downstream of oncogenic Ras during thyroid transformation, with an inverse correlation with miR-29b expression (see figure on the side), thus suggesting a new Ras-miR-29b-PATZ1 axis in thyroid carcinogenesis. Here, we investigated whether the down-regulation of PATZ1 plays a causal role in Ras-induced thyroid transformation. Elucidation of the molecular pathways downstream of oncogenic Ras, which are crucial for thyroid transformation, will help to find new therapeutic targets in thyroid cancer carrying Ras mutations.

Results

1. Restoration of PATZ1 expression in FRTL5-Ras cells

Western blot analysis of PATZ1 in cell clones and mass populations (MP) obtained by transduction of either a PATZ1-expressing plasmid (P1) or the empty vector (G). Vinculin expression has been analyzed as a loading control.

2. PATZ1 expression in Ras-transformed thyroid cells inhibits proliferation

Growth curves (mean values ± SE of three different clones) performed on PATZ1-expressing (FRTL5-Ras-PATZ1) compared to empty vector-transfected (FRTL5-Ras) and normal control (FRTL5) cells. *P < 0.05; **P < 0.01, as assessed by unpaired T test.

3. PATZ1 expression in Ras-transformed thyroid cells inhibits wound closure

Conclusions

- In RasR12-expressing FRTL5 cells, PATZ1 is downregulated.
- Expression of miR-29b, which targets PATZ1, is inversely correlated to PATZ1 during Ras-induced thyroid transformation in FRTL5 cells.
- PATZ1 transfection in RasR12-expressing FRTL5 cells is able to partially revert their transformed phenotype in terms of:
  - proliferation;
  - migration;
  - tumorigenicity.

All in all these results suggest that PATZ1 is a pivotal regulator acting downstream of Ras (likely via miR-29b) to suppress thyroid cell transformation driven by oncogenic Ras, highlighting a new potential therapeutic target to fight highly malignant thyroid cancer.

References: