The Impact Of Rab7 Expression In Head And Neck Squamous Cell Carcinoma Progression

Fernanda Giudice (A.C. Camargo Cancer Center, Brazil), Bruna Rodrigues (A.C. Camargo Cancer Center, Brazil), Tonielli Lacerda (A.C. Camargo Cancer Center, Brazil), Antuani Baptistella (A.C. Camargo Cancer Center, Brazil), Marcos Dias (A.C. Camargo Cancer Center, Brazil), Luiz Kowalski (A.C. Camargo Cancer Center, Brazil), Vilma Martins (A.C. Camargo Cancer Center, Brazil).

BACKGROUND: Recent evidences have shown that the tumor development and the metastatic progression are supported by exosomes, which are released from the tumor cells to the blood or lymphatic system. The cellular secretion of exosomes represents a significant system for short and long-range cell-to-cell communication. In this way, it is essential to understand the molecular mechanisms involved in exosome biogenesis and secretion. The Rabs are a group of GTP-binding proteins implicated in the secretory and endocytic pathways of eukaryotic cells.

HYPOTHESIS: Studies have pointed that Rab7, a GTPase that regulates trafficking of lysosomes, is involved in many disease states including cancer. Herein, we sought to evaluate the role of Rab7 protein in the regulation of exosome release and in the aggressive potential of head neck squamous cell carcinoma (HNSCC).

METHODS: Exosome derived from HNSCC cell lines (SCC-9, Cal27 and FaDu) were isolated by ultracentrifugation and quantified using Nanoparticle Tracking Analysis (NanoSight®). A screen targeting for Rab7 protein was also performed through western blotting and the lysosomal compartment was followed by immunofluorescence. Plasmid mCherry-Rab7 and GFP-Rab7-DN (dominant-negative) were used to overexpression and inhibit the activity of Rab7, respectively. After Rab7 modulation, it was evaluated the exosome secretion and the invasion capability of the cell lines. Finally, analyses of Rab7 expression in human clinical specimens (tissue microarray samples – 153 cases from tongue and floor of mouth) were performed through immunohistochemistry.

RESULTS: Comparing the three cell lines, FaDu cells secreted less exosomes than SCC-9 and Cal27. Remarkably, FaDu cells presented higher expression of Rab7. Immunofluorescence experiments showed the presence of numerous perinuclear clusters positive for LAMP-1 (lysosome marker) in FaDu and Cal27 cells, while SCC-9 showed LAMP1-positive organelles dispersed throughout its cytoplasm. These findings indicate that the majority of exosomes formed by FaDu cells can be driven to lysosome compartment instead of being released to the extracellular milieu. Functional assays showed that in cells overexpressing Rab7, the invasion pattern as well as the number of secreted exosomes is lower than in parental cells. On the other hand, the inhibition of Rab7 activity through overexpression of Rab7-DN increases the exosome release. Immunohistochemistry analyses revealed that HNSCC from patients that showed poor prognosis (cancer recurrence or cancer-related death during 170 months of follow-up) had significantly lower Rab7 protein expression than those patients that exhibited good outcome (disease-free survival for more than 60 months or non–cancer-related death with a maximum follow-up of 121 months). A significant overexpression of Rab7 was predictive of better survival (log-rank test, $p = 0.003$). Our data highlights a role for Rab7 protein in the modulation of exosome secretion by HNSCC. Moreover, the functional assays and immunohistochemistry results suggest the clinical importance of regulating Rab7 expression for the controlling of tumor progression and aggressiveness.