ID: 01045
Type: Poster
Topic: Tumor biology

Exosome biogenesis in the triple negative breast cancer cell line MB-MDA231 cells relies on the integrin beta 3 mediated endocytosis

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Introduction:

Tumors are not isolated units, but complex systems where cell-cell communication between intra-tumoral and extra-tumoral cells play a crucial role triggering tumor progression and metastasis. Apart from direct cell-cell contacts several mechanisms including communication through extracellular vesicles (EVs) have been described until now. EVs have been proposed to act as mediators of intercellular communication in physiological and pathological scenarios. EVs are membrane-limited vesicles secreted by normal and malignant cells and their function is dependent on the cargo they carry and the cell type from which they originate. They are classically defined by size and origin into two groups: exosomes and microvesicles. Exosomes are endosomal derived vesicles with a defined size of 30-150 nm. In contrast to exosomes, microvesicles or detaching vesicles, are a heterogeneous population of larger vesicles (0.1 – 1 mm) shed from the plasma membrane.

Endocytosis of integrins has been recognized several years ago and it is now well established that integrins are constantly endocytosed and recycled back to the plasma membrane through multiple routes. The pathways that regulate endocytosis and recycling of integrins have therefore arose as major players in controlling integrins activity. The tight regulation of integrin turnover has been shown to be crucial for a number of biological processes, including cell migration and cytokinesis. Importantly, accumulating evidence gathered over recent years clearly indicate, that endocytosis and recycling of integrins plays a crucial role during cancer progression, invasion and metastasis.

While it is known, that integrins are crucial for the role of exosomes in cancer progression and particularly in metastasis, the underlying molecular mechanisms remained largely elusive. Here we describe a fundamental link between endocytosis mediated integrin trafficking and the integrin mediated uptake of exosomes from the extracellular environment.

Objectives:

While it is known, that integrins are crucial for the role of exosomes in cancer progression and particularly in metastasis, the underlying molecular mechanisms remain largely elusive. To gain further insights, we were aiming at delineating the role of ITGB3 in exosome biogenesis in the triple negative breast cancer cell line MB-MDA231.

Methodology:

The work is based on studies in the triple-negative breast cancer cell line MDA-MB-231and stable shRNA mediated knockdown of ITGB3 was used to study its function.

Extracellular vesicles were isolated by ultracentrifugation and characterized by Western blot analysis to determine the protein composition and by Nanosight and CryoEM to measure the amount of secreted vesicles.

A mass spectrometry based survey was performed, to determine the differences in the protein composition of vesicles after knock-down of ITGB3.

Uptake of fluorescently labeled exosomes into recipient cells was measured by flow-cytometry.

Results:

Cells expressing a stable knockdown of ITGB3 showed a higher concentration of exosomal protein, as well as a higher amount of vesicles in the extracellular medium, analyzed either by Nanosight or and Cryo-EM.

Experiments with stable ITGB3 knockdown MDA-MB-231 cell line revealed that exosome uptake depends on ITGB3 expression in recipient cells, suggesting their important role in exosomes-mediated endocytosis.

Deficient exosomes uptake occurring in cells carrying ITGB3 knockdown, leads problems in exosomes biogenesis, reflected in the lack of classical exosomes markers, such as TSG101, CD81, but not others like Flotillin-1.

Mass-Spec analysis of shITGB3-MDA-MB-231 derived-exosomes showed a clear affectation of components from the well-known exosome formation ESCRT complex, as well as in the Syndecan-syntenin-ALIX, the major player controlling the formation of endosomal intraluminal vesicles that get released as exosomes, and the sorting of cargo in these vesicles.

Conclusions

Here we demonstrate that ITGB3 plays a crucial role regulating exosomes uptake driven by endocytosis and the consequence of this lack of vesicles recycling trafficking is the affection of exosome biogenesis.

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