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Role of NSCLC exosomes in osteoclast differentiation

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Bone metastases represent a frequent cause of morbidity and mortality in patients suffering many types of cancer including breast, kidney, and lung. Non-small cell lung cancer (NSCLC) is one of the most commonly diagnosed neoplasia. About 70% of NSCLC patients develop bone metastasis during the course of disease or at diagnosis. NSCLC frequently induces osteolytic metastases associated to bone lesions or destruction. NSCLC cells induce the release of factors that alter the regulatory networks existing between osteoblast and osteoclasts, modifying the normal balance of the receptor activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG). RANKL is essential during the differentiation stage from the preosteoclast
to active osteoclast. RANKL activity is counteracted by OPG that blocks the bind of this latter to its receptor RANK, thus preventing the RANK/RANKL signaling. RANK/ RANKL system can be modulated by epidermal growth factor receptor (EGFR) expressed in the preosteoclasts.
In NSCLC cells EGFR is overexpressed (1), the total effect is an of RANKL upregulation with a induction of osteoclast formation (2). EGFR binds several ligands among which AREG, a molecule overexpressed in several cancers such as colon, breast, and lung. Particularly, high level of AREG has been reported in exosomes released by cancer cells resulting in an increase of the invasiveness. Exosomes, released from cells under both physiological and pathological conditions, have recently been recognized as mediators in the cell-to-cell communication processes as they contain a variety of cargo such as mRNA, microRNA, DNA, and proteins (3). They seem to have a key role in the crosstalk in the tumor microenvironments promoting tumor growth and metastatic dissemination of a primary tumor. Our data report about the effects of NSCLC exosomes in the induction of pre-osteoclast
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differentiation to mature osteoclast, focusing on the role of AREG in the EGFR pathway activation and bone metastasis induction. The treatment of murine monocyte cells RAW246.7 with NSCLC exosomes enriched in AREG, modulates the typical phenotype of mature osteoclasts via EGFR pathway that in turn causes an upregulation of RANKL. Exosomes released in CRL-2868 cells conditioned media and in plasma of NSCLC patients, were isolated and morphologically and biochemically characterized. The effect of these exosomes on the RAW246.7 cell was also evidenced by morphological observations. Findings here reported show that RAW246.7 added of exosomes from NSCLC cell line CRL-2868, mature towards the osteoclastic lineage within 7 days from the treatment, resulting in multinucleated cells of various size and configuration. A better understanding of exosomes function can improve the therapeutic strategy to inhibit the attraction between lung cells and bone. In order to take advantage of exosomes as vehicles for delivering therapeutic reagent or drugs in the treatment of malignancies with bone osteolytic lesions.

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