52 miR-424 secreted in extracellular vesicles/exosomes promotes prostate tumorigenesis

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Introduction & Objectives: Prostate cancer (PCa) is the most commonly and frequently diagnosed malignancy in men. Metastatic and castration-resistance prostate cancer are the major clinical challenges in the management of patients with advanced disease. MicroRNAs are secreted in extracellular vesicles/exosomes exerting autocrine and paracrine functions in tumorigenesis with potential impact on metastasis and treatment resistance. miR-424 is highly expressed in prostate tumors and sustains cancer stem cell expansion and tumor progression. The goal of this study was to assess whether miR-424 secreted in exosomes could impact on transforming phenotypes and tumor development by an autocrine/paracrine mechanism.

Materials & Methods: We used parental prostate epithelial normal (RWPE-1) and cancer (LNCaP) cells and derivative cell lines engineered to stably express miR-424. Exosomes were isolated from cell culture medium and tumor xenograft tissues using miRCURY Exosome isolation kit or ultracentrifugation. Exosomal miR-424 level was measured by q-RT-PCR. Functional changes in the phenotype of recipient cells (parental RWPE-1 and LNCaP) were assessed using tumor-sphere formation, anchorage-independent growth and in vivo tumor formation assays. Exosomes were fluorescently labeled with PKH26 and detected by confocal microscopy for monitoring in vitro cellular uptake. For in vivo tissue distribution, exosomes were fluorescently labeled with DiD and detected by in vivo imaging with IVIS Spectrum. The effects on in vivo tumor growth were determined by a) pretreating in vitro recipient cells with control and miR-424 enriched exosomes before implantation in NSG mice and b) by tail vein injection of control and miR-424 enriched exosomes in NSG mice with preformed xenografts of recipient cells.

Results: miR-424 was released in culture medium and exosomes isolated from miR-424 expressing cells. Notably, exosomal miR-424 was also detected in the extracellular tumor microenvironment and in blood of mice bearing xenografts of miR-424 expressing tumors. Exosomal miR-424 was efficiently taken up by recipient RWPE-1 and LNCaP cells as shown by confocal microscopy and qRT-PCR. Exosomes containing miR-424 promoted in vitro transforming phenotypes, including expansion of stem-like tumorigenic cells and in vivo tumorigenicity in NSG mice. Moreover, systemic supplementation of miR-424 enriched exosomes by tail vein injection in NSG mice enhanced in vivo growth of subcutaneous tumor xenografts.

Conclusions: Our data demonstrate that miR-424 is secreted in exosomes and exosomal miR-424 influences tumor growth when supplemented in vitro and in vivo. miR-424 released in exosomes, through its ability to influence stem cell expansion and the tumor microenvironment, can impact on tumor progression and metastatic spread of prostate cancer. These findings can be exploited to design innovative therapeutic strategies for treatment of prostate cancer.