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Informations

Original article

Colorectal cancer cell–derived exosomes containing miR-10b regulate fibroblast cells via the PI3K/Akt pathway

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Summary

Background > Cancer-associated fibroblasts (CAFs) contribute to the proliferation of colorectal cancer(CRC) cells. However, the mechanism by which CAFs develop in the tumor microenvironment remains unknown. Exosomes may be involved in activating CAFs.

Methods > Using a miRNA expression profiling array, we determined the miRNA expression profile of secretory exosomes in CRC cells and then identified potential miRNAs with significant differential expression compared to normal cells via enrichment analysis. Predicted targets of candidate miRNAs were then assessed via bioinformatics analysis. Realtime qPCR, western blot, and cell cycle analyses were performed to evaluate the role of candidate exosomal miRNAs. Luciferase reporter assays were applied to confirm whether candidate exosomal miRNAs control target pathway expression. A CRC xenograft mouse model was constructed to evaluate tumor growth in vivo.

Results > Exosomes from CRC cells contained significantly higher levels of miR-10b than did exosomes from normal colorectal epithelial cells. Moreover, exosomes containing miR-10b were transferred to fibroblasts. Bioinformatics analysis identified PIK3CA, as a potential target of miR-10b. Luciferase reporter assays confirmed that miR-10b directly inhibited PIK3CA expression. Co-culturing fibroblasts with exosomes containing miR-10b significantly suppressed PIK3CA expression and decreased PI3K/Akt/mTOR pathway activity. Finally, exosomes containing miR-10b reduced fibroblast proliferation but promoted expression of TGF-β and SM α-actin, suggesting that exosomal miR-10b may activate fibroblasts to become CAFs that express myofibroblast markers. These activated fibroblasts were able to promote CRC growth in vitro and in vivo.

Conclusion > CRC-derived exosomes actively promote disease progression by modulating surrounding stromal cells, which subsequently acquire features of CAFs.

Background

The tinteraction between a tumor and its microenvironment is complex and contributes to carcinogenesis and cancer invasion [1]. Although most studies have indicated that cancer-associated fibroblasts (CAFs) drive cancer metastasis through paracrine regulation of biological processes in cancer cells, some researchers have argued against this pro-tumorigenic effect. In certain pancreatic cancer studies, fibroblasts have been shown to exert an anti-tumor function, whereby the removal of fibroblasts from the tumor promoted aggression and was accompanied by a poor prognosis [2]. Regardless, not all fibroblasts contribute to cancer progression, and they participate in cancer progression only after being activated, i.e., becoming CAFs. Raghu Kalluri suggested that 80% of stromal fibroblasts in a tumor acquire an activated phenotype to become CAFs [3]. CAFs produce and release cytokines, including transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8, and miRNAs that target pathways in cancer cells and promote cancer progression [4,5]. However, it remains unclear how fibroblasts become activated. In this study, we hypothesized that cancer cells induce fibroblast differentiation in the tumor microenvironment via exosomes, contributing to tumor progression.

An important type of cell-cell communication occurs through exosomes. These small, nanometer-sized (50–100 nM) vesicles of endocytic origin are released into the extracellular milieu by cells under physiological and pathological conditions, including antigen presentation and infectious agent transmission. By sharing genetic information or functional proteins to modulate cell behavior, tumor exosomes are important mediators of the cross-talk between tumor cells and their microenvironment [6,7]. miR-10b is a miRNA that has been detected in exosomes released by multiple types of cancer, including colorectal cancer (CRC) [8,9], breast cancer [10], and lung cancer [11]. Moreover, in CRC, miR-10b modulates the PI3K/Akt/mTOR pathway by targeting HOXD10, and high miR-10b expression is important for cancer cell invasion [8].

CRC is one of the most common malignancies worldwide, with 1.5 million newly diagnosed cases annually [12]. The 5-year relative survival rate is 90% for patients with localized disease and 13% for those with distant metastases at diagnosis [13]. Associated with poor prognosis, PI3K activity is significantly increased in CRC [14], often due to genetic aberrations such as growth factor overexpression and gene mutation. PI3K activation in vitro drives the transformation of well-differentiated epithelial cells to a less differentiated and more malignant phenotype [15], and mouse models of ulcerative colitis have revealed that PI3K activity is critical for progression to cancer [16]. However, much research to date has been focused on the function of PI3K signaling in tumor cells, whereas the effects of PI3K signaling in CAFs remain unclear. Moreover, it is unknown whether exosomes containing miRNAs can be released from cancer cells into the tumor microenvironment and then transferred to fibroblasts to induce phenotypic change in these target cells. In this study, we found that CRC cells secrete exosomes containing multiple miRNAs, including miR-10b. Moreover, exosomal miR-10b from CRC cells can be transferred to fibroblasts. Exosomes containing miR-10b promoted tumor growth and increased expression of TGF-β and SM α-actin in fibroblasts, which is characteristic of CAFs or fibro-myofibroblast differentiation. In addition, we investigated the pathway affected by miR-10b and found PIK3CA activity to be reduced in fibroblasts containing miR-10b. These findings indicate that cancer cells can control fibroblasts via exosomes, potentially representing a novel mechanism of cancer invasion or metastasis.

Materials and methods

Cell culture

The CRC cell line HCT116, normal human fibroblasts CRL1554 and 293T cells were purchased from American Type Culture Collection (ATCC) and maintained in EMEM (Invitrogen) or DMEM (HyClone), both of which were supplemented with 10% ultracentrifuged fetal bovine serum (FBS; Invitrogen), 1 mm sodium pyruvate (Invitrogen), penicillin (100 U/mL; Invitrogen) and streptomycin (10 mg/mL; Invitrogen) at 37°C in a humidified atmosphere with 5% CO_2. Trypsin (0.05%) and 0.02% ethylenediamine tetra-acetic acid (EDTA; HyClone) were also used in cell culture.

Human tissue and primary cell culture

Study protocols involving human materials were approved by the First Hospital of Shijiazhuang City Institutional Ethics Committee. Patients with CRC were involved in this study, and we obtained informed consent from 17 patients who underwent a radical colectomy. However, samples from only 5 patients were included for the following reasons:

- we could not obtain specimens from 3 patients;
- we could not culture cells from 8 patients;
- one patient withdrew consent.

Medical charts were comprehensively reviewed. Patients receiving current radiation treatment and those receiving chemotherapy before surgery were excluded. Frozen slides of specimens were examined by a pathologist, and the areas of cancer and adjacent normal tissue were labeled. Tissues were obtained from specimens via needle biopsies taken from the labeled tumor and adjacent normal tissue areas under direct supervision of a pathologist.

The fresh biopsy tissue was minced into small 1-mm^3 pieces in 12.5 mL of DMEM supplemented with 0.06% collagenase A (Sigma) and incubated overnight; 12.5 mL 0.05% trypsin and 0.02% EDTA were added for 1 h at 37°C with 5% CO_2. The cell pellets were then collected, washed in phosphate-buffered saline (PBS), and seeded into a 10 cm culture dish under