Extracellular Vesicles from a Novel Chordoma Cell Line, ARF-8, Promote Tumorigenic Microenvironmental Changes When Incubated with the Parental Cells and with Human Osteoblasts

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Background

- Chordomas are extremely rare tumors of the sarcoma group; nonetheless, they are the most common tumor of the sacral and cervical spine. These tumors are notoriously resistant to therapies.
- Exosomes – secreted vesicles with multifaceted activities – are involved in tumor communication and material exchange in the tumor microenvironment.
- There are no published reports on the presence, contents, and effects of exosomes in chordomas.
- This provides an opportunity for novel exploration of the effects of exosomes on chordoma cells and the normal cells of their microenvironment, osteoblasts.
- We suspect both chordoma exosome-exposed ARF-8 chordomas and chordoma exosome-exposed osteoblasts will experience alterations to signaling, metabolism, and secretion of modifying material in the extracellular matrix.

Methods

- ARF-8 chordoma cells were grown in DMEM+10% exosome-depleted FBS.
- Conditioned medium underwent differential centrifugation, ultrafiltration, and ultracentrifugation to acquire ARF-8 exosomes.
- Purified ARF-8 exosomes were applied to ARF-8 chordoma and osteoblast cells (in triplicate, respectively) while control chordoma and osteoblast remained untreated (PBS). Both sets of triplicate were incubated at 24 hours.
- After incubation, the medium was removed and the cells were washed in ice-cold PBS (x3), then removed and centrifuged (@17k, 20mins, 4C).
- Both control and treated chordoma and osteoblast triplicates underwent proteomic, secretome, and pathway analysis.
- The removed conditioned media underwent secretome analysis.

Conclusions

- Proteomic analyses suggests roles for transforming growth factor beta (TGFB/TGFβ), cell–matrix interactions involving the epithelial-to-mesenchymal transition (EMT), and cell-extracellular matrix interactions in cell migration, consistent with a migratory/metastatic tumor phenotype.
- ARF-8 tumor cell migration was dependent on general (arginine–glycine–aspartic acid [RGD]-based) integrin activity and that ARF-8 EVs could promote such migration.

Acknowledgements

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Add'l Figures & References

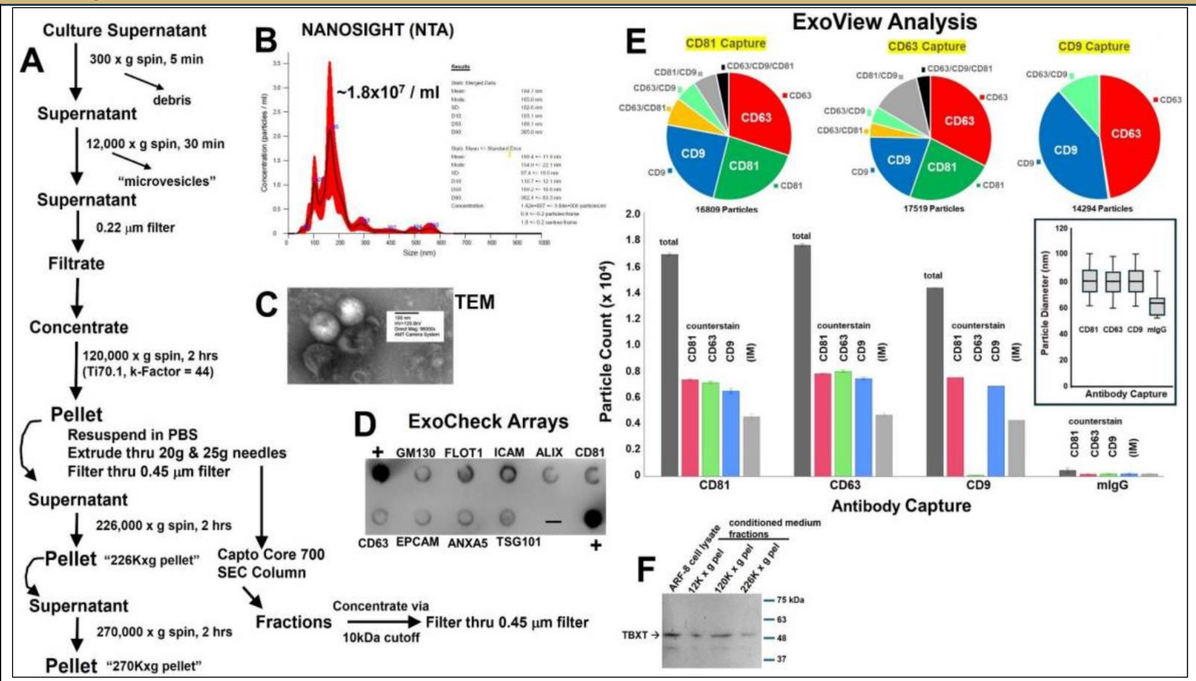

Figure 1 [Above]. A) Schematic of steps involved in EV isolation. **B)** Nanosight nanoparticle tracking analysis of ARF-8 EVs. **C)** Transmission Electron Microscopy of ARF-8 EVs. **D)** ExoCheck Arrays showing putative typical EV markers for lysed EVs from the ARF-8 cell line. **E)** ExoView single-particle interferometric reflectance imaging sensing (SP-IRIS) with immunofluorescence counterstain of CD81, CD63, and CD9 markers on immunocaptured EVs. **F)** Western blots of ARF-8 cell lysates and fractionated conditioned media extracellular pellets probed with anti-brachyury/TBXT antibody.

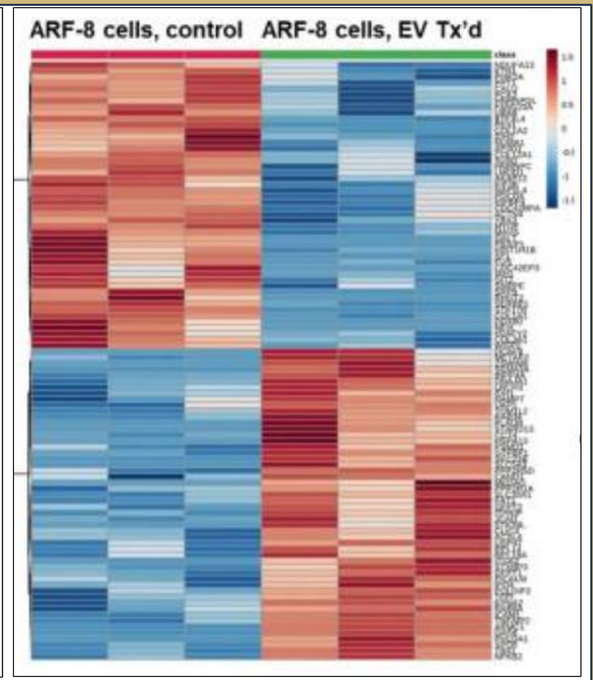

Figure 2 [Above]. Hierarchical clustering ARF-8 heatmaps from ANOVA statistical analyses utilized normalized data that were standardized by autoscaling features (top 100) with Euclidean distance measurements and clustered by ward; control ARF-8 cells, left; EV-treated ARF-8 cells, right.

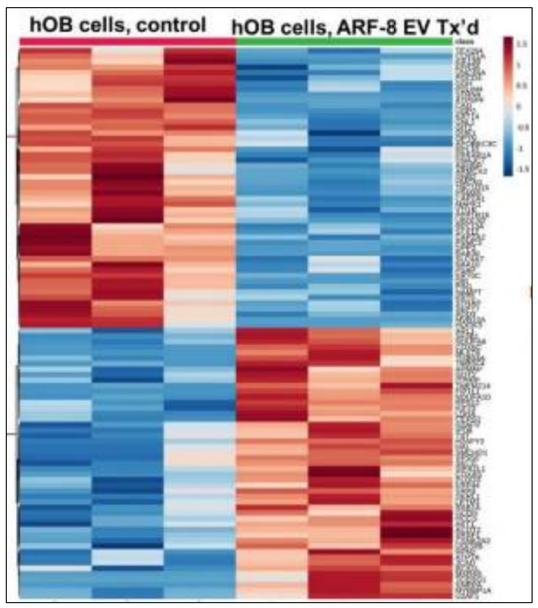

Figure 3 [Left]. Hierarchical clustering hOB heatmaps from ANOVA statistical analyses utilized normalized data that were standardized by autoscaling features (top 100) with Euclidean distance measurements and clustered by ward; control hOB cells, left; EV-treated hOB cells, right.

Figure 4 [Right]. A) Scratch closure assays with ARF-8 cells migration under control versus RGD. B) Migration assay of ARF-8 cells at various EV concentrations. C) Western blot probing for TGFB-1 of fractionated ARF-8 conditioned medium showing reactivity in high-speed centrifuged pellets.