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Exosomes, small extracellular vesicles carrying lipids, proteins, DNA and RNA, enable intercellular communication. Pituitary-derived exosomes have not been well validated, and as no human pituitary cell lines are available, we characterized exosomes derived from rat somatotroph tumor cells (GH1 and GH3). Rat FR and H9C2 cells were used as non-pituitary controls. Exosomes were isolated from serum-free culture supernatants by combining ultrafiltration and ultracentrifugation to eliminate hormone contamination. Derived exosomes were analyzed by NanoSight to visualize, size, and count particles. Exosomal proteins were extracted and exosome markers including TSG101, ALIX, CD63, HSP70, HSP90 detected by Western Blot. The exosome inhibitor GW4869 (10 μM, 30 h) reduced exosome release (up to 81%), whereas treating cells with hydrocortisone (0.1 μM, 72 h) increased exosome production (up to 42%) in GH1 and GH3 cells. Exosomal shuttle RNA characterized by RNA-Seq showed distinct pituitary vs non-pituitary exosome RNA profiles. Selected miRNAs assessed in exosomes and corresponding cells by qRT-PCR validated exosomal RNA-seq and suggested that miRNA signatures in exosomes and in respective cells of origin were concordant. Next, we explored downstream signaling of GH1-derived exosomes (GH1-exo) *in vitro* and *in vivo* and studied biological actions in normal hepatocytes and in malignant cells. As evidenced by mRNA-seq, GH1-exo distinctly altered signaling pathways in rat primary hepatocytes, vs pathways elicited by GH or PRL (0.5 μg/mL, 24 h). GH1-exo, FR-exo or vehicle were intravenously injected to 4-week-old female Wistar rats twice weekly for 4 weeks ($5*10^9$ exo/200 g, n=3), and livers dissected for mRNA-seq. Among GH1-exo specifically regulated genes, EIF2AK/ATF4, involved in cAMP responses and amino acid biosynthesis, were attenuated. In hepatocytes, GH1-exo suppressed up to 65% of nascent protein synthesis and reduced forskolin (10 μM)-stimulated cAMP activity by 19%, while GH (0.01-1 μg/mL) did not affect this pathway. Notably, GH1-exo also attenuated malignant cell motility. Both GH1-exo incubation or GH1 cell co-culture (48 h) suppressed migration, invasion and wound healing of HCT116 cancer cells by up to 70%. In contrast, treatment with rGH (0.5 μg/mL) increased HCT116 motility. Intravenous administration of GH1-exo (10^{10} exo/mouse, twice a week for 5 weeks) decreased metastatic tumor volume by 40% in nude mice harboring splenic HCT116 implants ($5*10^5$ cells/mouse, n=10), and especially abrogated hepatic metastases. mRNA-seq of GH1-exo treated HCT116 cells vs controls indicated dysregulated p53 and MAPK pathways, which may partially explain mechanisms underlying motility attenuation. The results elucidate novel biological actions of somatotroph adenoma cell-derived exosomes and suggest exosomes as non-hormonal messengers produced by pituitary tumors.

Neuroendocrinology and Pituitary

TOOLS AND MECHANISMS OF REGULATION IN THE ANTERIOR PITUITARY

Single Nucleus Transcriptome and Chromatin Accessibility Landscapes of Human Pituitaries

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The pituitary gland regulates key physiological functions, including growth, sexual maturation, reproduction, and lactation. Here, we present a paired single-nuclei (sn) transcriptome and chromatin accessibility characterization of six *post-mortem* human pituitaries. These samples were from juvenile, adult, and elderly male and female subjects. Well-correlated snRNAseq and snATACseq datasets facilitated robust identification of the major pituitary cell types in each sample. Using latent variable pathway analysis, we uncovered previously unreported coordinated gene expression modules and chromatin accessibility programs for each major cell type as well as an age-specific program across all the endocrine cell types. These largely appear to be congruent between human and mouse datasets. Given the importance of murine models in the study of human pituitary disorders and pituitary physiology, we next sought to compare expression profiles of pituitary cell types in mouse vs. human. Murine and human cell types were well correlated, exemplified by coordinated gene expression programs, especially for undifferentiated stem cells (SCs). In both species, we identified clusters corresponding to naive and committing SCs. All human SC clusters expressed the established SC markers *SOX2* and *SOX9*, as well as genes involved in SC regulatory pathways (*WWTR1*, *YAP1* and*PITX2*). Additional markers previously reported in murine pituitary SCs were also found in human SC, including *WIF1*, *LGR5*, *FOS*, *CDH1*, *EGFR*, *LGR4*, and *WLS*. Remarkably, in human, the main naive SC cluster was roughly divided into a high-*JUN* and a low-*JUN* expressing subgroup, whereas *Jun* expression was less pronounced in the murine SC cluster. In both species, committing SC clusters expressed the endocrine markers for *POU1F1*, *TSHB*, or *POMC*, while SCs committing to an intermediate lobe/melanotrope cell identity were distinguishable based on *PAX7* expression. In addition, in the human datasets we identify a population of cells as originating from the *pars tuberalis*. We offer a range of markers that can be utilized for *in vivo* validation of these cells. Overall, the characterization of the murine and human pituitary SCs strongly suggests the co-existence of subpopulations with different lineage commitments in addition to a single uncommitted SC population. This sn atlas of the human pituitary is a valuable resource that will be made web-accessible.