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Exosomes Isolated from Conditioned Media of Immortalized Kisspeptin Neurons Exert Diverse Effects on Central and Peripheral in Vitro Cell Models.

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Previous work in our laboratory explored the proteomic contents of exosome-like extracellular vesicles (EVs) released into the media in vitro from immortalized Kisspeptin (KP) neuronal cell lines KTaV-3 (derived from female mouse AVPV) and KTaR-1 (derived from KNDy neurons in the ARC). EVs were isolated from conditioned media via ultracentrifugation or filtration kit and validated using a NanoCyte, and LCMS-MS analysis revealed that relative abundance of exosomal cargo varied dependent upon estrogen (E2) exposure in vitro, with ~150-170 proteins up-regulated and ~200-220 proteins downregulated by E2 in EVs of KTaR-1 and KTaV-3 KP neurons.

Since E2-regulated KP exosomal proteins included candidates implicated in the regulation of synaptic plasticity and signaling (i.e. annexins, semaphorins, connexins), we investigated the effects of exposure to purified EVs on gene expression in immortalized GnRH neurons (GT1-7 cells). Notably, EVs from 24h E2-treated KTaV-3 neurons induced increased expression of kiss1r in GT1-7 cells, in contrast to KTaV-3 neurons not exposed to E2, suggesting that AVPV KP neurons may signal an increase in KP receptivity in GnRH neurons in vivo via non-neuronal communication. Additionally, increases in expression of the synaptic scaffolding protein PSD-95 (dlg4) were seen in GT1-7 cells treated with E2-treated KTaV-3 EVs, suggesting AVPV KP neurons may use extracellular vesicles to modulate GnRH neuronal synaptic plasticity over the estrous cycle.

While EVs isolated from KTaR-1 conditioned media did not alter GT1-7 kiss1r or dlg4 levels, treatment (24h) resulted in induction of selective gap junction hemichannel expression in GT1-7 cells. KTaR-1 (but not KTaV-3) EVs increased expression of Cx26 (gjb2) irrespective of E2 exposure, while induction of Cx43 (gja1) expression in GT1-7 cells was only observed following treatment with E2-deprived KTaR-1 EVs.

Further, we found that KTaR-1 media and EVs can affect osteoblast function in vitro, including increases in sp7 and runx2 expression, E2-dependent modulation of wnt10b, and formation of bone matrix (evaluated by Alizarin Red assay) in cultured osteoblast lines, supporting recent studies implicating ARC KP neurons in bone remodeling. Lastly, we found significant levels of immunomodulatory pentraxins (PTX3) in KP EVs, with abundance dependent upon prior E2 exposure in KTaR-1 KP neurons, as revealed by ELISA. Together, results from these studies suggest that EVs may represent additional intercellular communication pathways utilized by Kiss-1 neurons to elicit changes in nearby neuronal populations and potentially even in the periphery, affecting inflammation and bone remodeling. Future studies will address mechanisms involved in the E2 regulation of exosomal cargo in these critical neuronal populations.

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