Glioblastoma Cell Cilia Release Ectosomes Containing Signaling Factors That Promote Cell Proliferation

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Abstract
The mechanisms by which glioblastoma (GBM) cell primary cilia influence tumor pathogenesis are unclear. Primary cilia were originally viewed as exclusively sensory organelles. Recently, in non-cancerous cells, they have been shown to release ectosomes from their tips, vesicles which contain matrix metalloproteinases, G protein-coupled receptors, signaling mediators (e.g., Arl13b), and transcription factors that promote cell proliferation. In the present study, we analyzed GBM cell cilia formation and behavior and assessed the effects of conditioned media derived from ciliated or non-ciliated GBM cell cultures on GBM cell proliferation. Using piggyBac technology, we generated several GBM patient-derived cell line clones that stably express Arl13b:GFP and whose cilia are GFP-positive. Live imaging of Arl13b:GFP-positive GBM cilia from several clones revealed that some of these cilia release vesicles from their tips into the extracellular milieu. Interestingly, we found that the ciliary ectosomes are Arl13b-positive but acetylated alpha-tubulin-negative for both human and mouse glioma cells. Recent studies have reported that ciliary excision occurs during the G0 phase of the cell cycle, prior to cilia resorption and cell cycle re-entry, and requires the recruitment of F-actin and actin regulators into the cilium. Our analyses of GBM patient biopsies, tumors of human GBM-implanted mice, and GBM cell lines revealed that cells bearing budding cilia are Ki67-negative and that scattered cilia express F-actin (usually not present in normal cell cilia), indicating that GBM cilia excision might also employ an actin network-based mechanism. Importantly, GBM cell proliferation was significantly increased when cells were grown in conditioned media derived from ciliated GBM cell cultures compared with media from cilia-depleted GBM cells. Furthermore, conditioned media from ciliated cells did not enhance the proliferation of cilia-depleted cells, suggesting that ciliated GBM cells release factors that only affect other ciliated GBM cells. Finally, we found that increased expression of ARL13b, which is a predictor of decreased overall patient survival in glioma, recruits smoothened (SMO) into the cilium and stimulates the rate of GBM cell proliferation, thus mimicking the effects of sonic hedgehog exposure. Our data suggest that the release of vesicles from GBM cell cilia represents a novel mode of intercellular communication that might contribute to GBM pathogenesis.