Encyclopedia

Cytonemes in Tumourigenesis

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Increasing evidence during the past two decades shows that cells interconnect and communicate through cytonemes. These cytoskeleton-driven extensions of specialized membrane territories have emerged as a novel alternative for cell to cell communication that are involved in development, physiology, and disease. Several recent studies have shown that signalling pathways mediated by cytonemes during development, are essential for certain tumoral cell types progression.

In *Drosophila* wing disc EGFR and RET tumour models, cytoneme formation is required to receive signals from the neighbouring cells. Genetic ablation of cytonemes prevents tumour progression, restores apico-basal polarity, and improves survival.

Furthermore, cytonemes in the *Drosophila* glial cells are essential for glioblastoma progression as they alter Wg/Fz1 signalling between glia and neurons. Research on cytoneme formation, maintenance, and cell signalling mechanisms will help to better understand not only physiological developmental processes and tissue homeostasis but also cancer progression.

The nature of these interconnecting structures and their similarities with epithelial cytonemes are currently under debate. Cytonemes have been proposed to mediate communication between neoplastic cells and cells in their microenvironment [1]. In a *Drosophila* wing disc tumor model utilizing ectopic expression of the *epidermal growth factor receptor* (*EGFR*) and *receptor protein-tyrosine kinase* (*Ret*) oncogenes, cytoneme formation is required to receive signals from the neighboring cells. Genetic ablation of cytonemes prevents tumor progression, restores apico-basal polarity, and improves survival [1]. This recently established system serves as an optimal platform for novel pharmaceutical approaches against cancer progression *in vivo*. The authors identified pharmacological combinations against cytoneme-mediated oncogenic signals that prevent tumor progression and improve life span. The value of flies as a valid platform for human disease has accumulated evidences that favor this model for future preclinical studies. In particular, the high cost of testing single or combined pharmacological treatments in mice is several orders of magnitude higher than *Drosophila* based platforms, which has made preclinical trials risky and challenging. The molecular basis underlying cytonemes, the signals transduced by cytonemes, and the implications in tumorigenesis are hot topics for human disease that open novel avenues for potential future treatments.

In addition, the discovery of tunneling nanotubes (TNTs) brings a novel class of thin and long membrane protrusions that connect benign tumor cells [2]. These protrusions form complex networks that mediate the selective transfer of vesicles, organelles, and small molecules [3][4]. TNTs are a common phenomenon in different cell types and tissues that increase under pathological conditions, such as infections, cancer, or neurodegenerative diseases [3]. One considerable limitation to the study of TNTs is the fragility of these structures that makes TNTs difficult to preserve after fixation of tissues. This brought a controversy about their existence *in vivo*. However, intravital techniques enabled the study of TNTs in live animals [5], which revealed that TNTs are indeed relevant cellular structures *in vivo*.

In vivo microscopy methods have been used in recent years to study in detail cellular features of cancer cells. A recent study showed that TNTs are induced by stress in prostate cancer and they had a role in mediating intercellular communication that confer stress adaptive cell survival and treatment resistance

on the tumoral cells [6]. Additionally, pancreatic cancer cells show TNTs and their formation is stimulated after chemotherapy exposure [7]. Furthermore, TNTs are involved in the communication between tumor cells and macrophages to promote macrophage-dependent tumor cell invasion both *in vitro* and in an *in vivo* zebrafish model [8]. Interestingly, colorectal cancer cells have the ability to form locomotory and invasive filopodia that promote invasion and metastasis, and this is suppressed by the phosphorylation of Vasodilator-Stimulated Phosphoprotein (VASP) [9]. Related to colorectal cancer, leucine-rich-repeat containing G-protein-coupled receptor 5 (Lgr5), which labels crypt stem cells, represents the cell of origin in gastrointestinal cancers [10], and Lgr5 promotes the formation of cytonemes in mammalian cells suggesting a possible role for cytonemes in gastrointestinal cancer cell survival, invasion, and metastasis [11]. Exo70, a key component of the Exocyst complex, induces extensive actin membrane protrusions resembling filopodia and blocking Exo70 function inhibits invadopodia formation [12]. Exo70 expression is upregulated in colon cancer samples and its expression is positively correlated with tumor size, invasion depth, and distant metastasis. Colon cancer patients with higher Exo70 expression have a poorer clinical outcome than those with lower Exo70 expression [13].

In particular, glioblastoma (GB) cells produce long cellular protrusions at the invasive edge of the tumor that scan the surrounding area and interconnect tumor cells. These protrusions are F-actin based and form a complex network that interconnects GB cells; therefore, they are named tumor microtubes (TMs) [14]. TMs contribute to invasion and proliferation, resulting in effective brain colonization by GB cells. Moreover, TMs constitute a multicellular network that connects GB cells over long distances (up to 500 µm length) [14]. This study found that Growth Associated Protein-43 (GAP43) is essential for the development of TMs and the tumor cell network associated with GB progression, and it drives TM-dependent tumor cell invasion, proliferation, interconnection, and radioresistance. TMs share many characteristics with cytonemes, they are actin-based projections and they are marked by several cytoneme markers, including Ihog, LifeActin, Moesin (GMA), glycosylphosphatidyl-inositol (GPI), myosin light chain (MLC), and the nonmuscle type 2 myosin, spaghetti squash (sqh). Moreover, this study [15] showed in a *Drosophila* glioma model that the glioma network is dependent on the fly *GAP43-like* gene (*igloo, igl*), as has been described in human tumor cells. The glioma network does not develop upon *igl* silencing. TMs stability in GB is sensitive to *GAP43* expression in tumoral cells. Moreover, downregulating *Neuroglian* (*Nrg*), which is known to prevent epithelial cytoneme formation, resulted in a reduction of the TM-like processes in GB [15]. Moreover, TMs accumulate Frizzled1 receptor (Fz1) that mediates Wingless (Wg) signaling (Figure 1) [15]. Thus, there are molecular and functional similarities between cytonemes and TMs; however, the term cytoneme is used for physiological situations, and TMs is restricted to the tumoral condition.

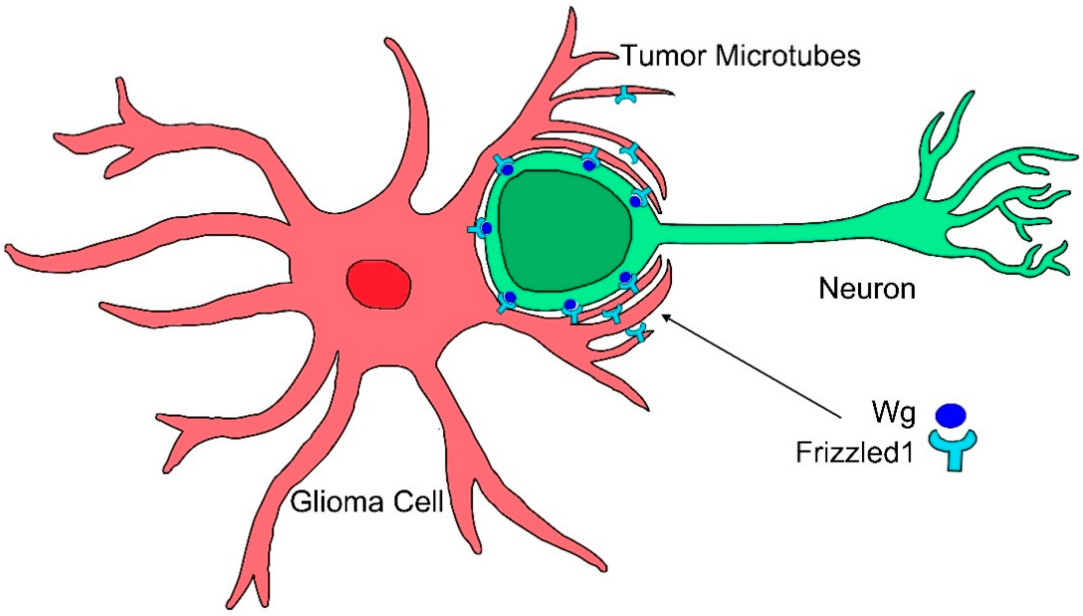

Figure 1. Cytonemes in tumourigenesis. Glioma cells produce a network of tumor microtubes that grow to reach and enwrap neighboring neurons. Increased glia–neuron membrane contacts facilitate neuronal Wg

sequestering mediated by glioma Frizzled1 receptor accumulated in the tumor microtubes [16].

TMs and TNTs share some structural features, but TMs are more stable, longer, and thicker (2 μm). In addition, TMs in human cells provide functional coordination to GB cells and facilitate cell repair, brain infiltration, and offer resistance to radiotherapy through dilution of Ca^{+2} intracellular peaks [14], which thereby increases the aggressiveness of GB.

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Keywords

Cytonemes; Drosophila; glioblastoma; tumourgenesis; cell signalling; cell-cell communication