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How to move a "fried egg": membrane blebbing in oligodendrogliomas

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Oligodendroglioma, astrocytoma, and glioblastoma are recognized as different tumor entities based on their histology, genetic makeup, and clinical outcome. Of the 3 glioma types, oligodendrogliomas display the most striking morphology in histological sections with a dark rounded nucleus within a white "empty" (delipidated) space, reminiscent of "fried eggs." Differences at the biological level are only beginning to be elucidated. In this issue of *Neuro-Oncology*, Demirdizin et al. provide insight into unexpected phenotypes and molecular players that are specific to oligodendrogliomas.[1]

Once again, tumors appear to draw on developmental processes for their functional needs. Tripartite motif-containing 67 (TRIM67), proposed here as a novel oncogene in oligodendrogliomas, is a ubiquitin ligase that attaches ubiquitin peptides to target proteins. Knockout mice of TRIM67 display developmental defects in specific brain regions as well as cognitive and behavioral impairment.[2] It has long been recognized that ubiquitination is not only involved in protein degradation but represents a versatile signal regulating multiple functions including axon guidance, cell signaling, and exocytosis.[3] The number of ubiquitin ligases, more abundant in the human genome than protein kinases, increased during evolution in particular with the complexity of the nervous system. In conjunction with another ubiquitin ligase TRIM9 (Tripartite motif-containing 9), TRIM67 positively regulates growth cone steering and filopodial dynamics through coordinated ubiquitination of cytoskeletal proteins such as VASP (Vasodilator-stimulated phosphoprotein) actin polymerase.[4] TRIM67 also interacts with the netrin receptor deleted in colorectal cancer and with exocytic proteins to regulate plasmalemma expansion and exocytosis in neurons. Here Demirdizin et al. report that TRIM67 mediates the alteration of glioma cell morphology towards a rounded shape and the formation of so-called membrane blebs. Membrane blebbing is recognized as a localized decoupling of the actin cytoskeleton from the plasma membrane, enabling enhanced cell locomotion and cell division.[5] It is also a well-recognized feature of cells undergoing stress and apoptosis.

In their study, the authors came across TRIM67 as one of the top upregulated genes in oligodendrogliomas compared to astrocytomas and glioblastomas. Overexpression of TRIM67 in glioma cells resulted in the activation of genes associated with cytoskeleton and adhesion such as paxillin, Rho GTPase, RAC, and integrin signaling. The effects were seen at the transcriptomic and protein level. Using coimmunoprecipitation, the authors confirmed the interaction of TRIM67 with several proteins, including TRIM9, the known antagonist of TRIM67 in axon branching and exocytosis in the normal brain. Phenotypically, TRIM67 overexpression led to F-actin aggregates and a more rounded morphology and membrane blebbing. Bleb formation was inhibited by TRIM67 knockdown as well as chemically by the myosin class II inhibitor blebbistatin and the ROCK inhibitor fasudil. In a series of functional assays, the authors show that TRIM67 may stimulate cell contractility via Rho GTPase RHOA and Rho-associated kinase (ROCK), via their upstream regulator NOGO-A (neurite outgrowth inhibitor A), a known myelin-associated axon growth inhibitory factor. At the functional level, TRIM67 overexpression reduced cell adherence and increased the migration potential in several cell lines. In orthotopic mouse xenografts, TRIM67 overexpression leads to larger tumors and a decrease in mouse survival.

Although some pieces of the puzzle are provided, mechanistically, it is unclear how and where TRIM67 acts to induce the observed effects. The full interactome of TRIM67 is not yet well understood.[6] While the authors focused on the interaction between TRIM67 and TRIM9 due to their known roles in axon guidance, the co-immunoprecipitation experiment also revealed a particular abundance of septins. As GTP binding proteins, septins are an important component of the cytoskeleton mediating cell shape and motility. Septins form protein complex rings, which serve as scaffolds for the recruitment of proteins from cytoskeleton and plasma membrane. As they are required for membrane retractions during blebbing and vesicle fusion,[7] it would be worthwhile to investigate their role in TRIM67-induced blebbing (Figure 1).

How to interpret the functional consequences of TRIM67 expression in oligodendrogliomas? Membrane blebbing is a phenomenon observed in various biological processes including cell division, morphological changes, and motility as well as

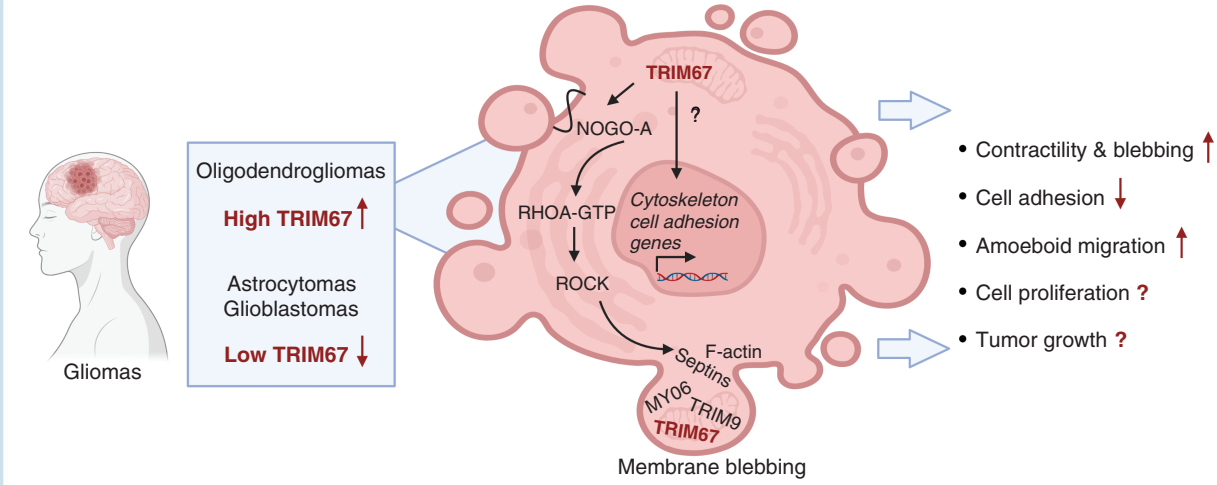

Figure 1. Schematic representation of the role of TRIM67-mediated membrane blebbing as described in the study of Demirdizen et al. (Illustration created with Biorender.com).

apoptosis. Although induction of apoptosis has been excluded, the impact on proliferation has not been directly assessed. While a direct link between TRIM67-mediated membrane blebbing and oligodendroglioma migration remains to be shown, it is tempting to speculate that TRIM67 may impact the mode of cellular migration. The common feature of diffuse gliomas is an extensive tumor cell invasion into brain parenchyma, yet different tumor entities may use distinct patterns of motion.[8] Astrocytoma and glioblastoma cells appear to advance in an elongated mode, often forming a network of migratory cells connected through thin membrane protrusions.[9] Interestingly, oligodendrogliomas do not form such cellular networks and may instead rely more on ameboid migration. This type of motility does not require matrix metalloproteinases to degrade the extracellular matrix. Bleb-driven ameboid locomotion relies on myosin II-dependent hydrostatic pressure leading to membrane protrusions which detach from the actinmyosin cortex at the leading edge of the cell. By squeezing membrane blebs through the matrix pores, cells physically deform the surrounding matrix and gain space for moving forward.

The overall significance and generalization of the findings need further investigation. Despite compelling in vitro data, surprisingly the in vivo experiment in Demirdizin et al. does not provide evidence of increased migration on TRIM67 overexpression. Another conundrum to solve is the observation that the Ki-67 index remains unchanged while the tumor mass appears to be significantly enlarged in TRIM67-expressing tumors. High TRIM67 expression in oligodendrogliomas has been confirmed in a recent study[10] and was also found in neuroblastomas and paragangliomas. Yet it is currently unclear whether all oligodendroglioma cells express TRIM67 or whether it is a distinctive feature of invading cells. It also remains to be seen whether the role of TRIM67 is specific to oligodendrogliomas or can be generalized to other cells. Along these lines, tumor or tissue-specific effects seem to add another level of complexity: in contrast to oligodendroglioma and neuroblastoma, TRIM67 has been attributed to a tumor-suppressive role in lung, colon, and gastric cancers. While many questions remain, Demridizin et al. added a compelling and unforeseen flavor to "fried eggs."

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