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Commentary

# Reprogramming of GBM microenvironment by large oncosomes: 'Traveling' V-ATPases are doing more than acidification

Delphine Garnier [a,b,*]

[a] CRCINA, INSERM U1232, Université d'Angers, Université de Nantes, Nantes, France
[b] Equipe Apoptose et Progression tumorale, LaBCT, Institut de Cancérologie de l'Ouest, Saint Herblain, France

Glioblastoma (GBM) is the most common and most aggressive form of adult primary brain tumor, and is uniformly fatal. Sadly, no improvement in patients overall survival has been observed since the establishment of the standard of care in 2005, that combines surgery to concomitant and adjuvant radiotherapy/chemotherapy [7]. Despite intensive research, the treatment of GBM is still facing different challenges, partly coming from the extremely heterogeneous and invasive phenotype of GBM tumors, drug resistance and invariable recurrence. All these features are related to the presence of Glioblastoma stem cells (GSCs), that interact with the tumor microenvironment called tumor niche [3] in part through the release of extracellular vesicles. Those vesicles can be transferred from donor to recipient cells at local or distant sites and include small exosomes, microvesicles, and large oncosomes (LOs) [5].

In the present manuscript Bertolini et al. investigate the capacity of GSCs to reprogram surrounding normal and tumor cells through the secretion of LOs in the extracellular milieu [1]. While the involvement of exosomes in GBM has already been described, the work presented in this issue opens new perspectives by focusing on V-ATPases (vacuolar H+-ATPases) and LOs. These ATP-dependent protons pumps allow the acidification of intracellular organelles and the extracellular space, but recently some interest has been growing towards the involvement of these enzymes in cancer progression as well as metastases formation, invasion and drug resistance [6].

As a follow-up of the previous study published on V-ATPases (Di Cristofori [2]), Bertolini et al. first made a connection between ATP6V1G1 expression and the upregulation of homeobox genes in GBM patients-derived orthotopic xenografts. The most remarkable result was the observation of a similar profile inside the LOs secreted by GSCs donor cells, as well as their transfer to recipient normal or tumor cells. As a consequence a long-term increase in the expression of homeobox genes HOXA7, HOXA10, POU3F2 as well as ATP6V1G1 was observed in recipient cells. This was paired to an increased tumorigenic potential, evidenced either by a higher rate of proliferation of non-neoplastic cells, or a greater capacity to invade and form neurospheres in glioma cells. This nicely did correlate to patient data, as circulating LOs isolated from the blood of GBM patients showed an increased level of POU3F2 and ATP6V1G1 compared to lower-grade glioma, and could also be incorporated into non-neoplastic margin cells. The authors therefore suggest the use of POU3F2 and ATP6V1G1 mRNAs from LOs as clinical blood markers to track glioma stage and evolution.

Finally, in order to precise the role played by V-ATPases in the processes described above, Bertolini and colleagues used Bafilomycin A1, a non-specific V-ATPase inhibitor, or siRNA against ATP6V1G1. Very interestingly, when replicating the previous experiments of LOs transfer from GSCs to non-neoplastic cells after treatment of donor cells with Bafilomycin A1, the increase in proliferation of non-neoplastic cells and in sphere formation of glioma cells were lost. While the neutralization of lysosomal acidification in donor cells with ammonium chloride reduced the clonogenicity and invasiveness of recipient cells exposed to LOs, homeobox gene expression was not modified suggesting that several mechanisms may explain the effects observed.

Overall the work presented in this manuscript is of particular interest for both scientific research and the treatment of GBM in the clinic. On the research side, this paper is bringing some light into the mechanisms controlling oncogenic transformation through the emission of extracellular vesicles. This may serve as a base for further studies to decipher the signaling involved in the increased potential in proliferation/invasion/sphere formation in normal as well as tumor recipient cells. In particular some future work could aim at exploring the respective roles played by acidification and non-canonical functions of V-ATPases. A comprehensive analysis of homeobox genes signaling may also help to understand how they contribute to the phenotype observed.

On the clinical side, Bertolini et al. were able to demonstrate that the detection of ATP6V1G1 and homeobox genes in LOs isolated from blood of GBM patients could become a powerful diagnostic tool to classify glioma stages, as further detailed in a companion paper published in the same issue [8]. On the other hand, this study highlights the relevance of developing new therapeutic strategies based on the targeting of V-ATPases, while taking advantage of the inhibitors already used in clinics. Indeed, optimization of such a therapy could benefit from the broad use of protein pump inhibitors (PPI), the repurposing of those drugs being already investigated for other types of cancer [4]. The limitation may however come from the necessity to find very specific inhibitors, considering the great diversity of V-ATPases and their contribution to a wide variety of normal processes. Also, as suggested by the

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* CRCINA, INSERM U1232, LaBCT ICO Site René Gauducheau, Boulevard Professeur Jacques Monod, 44805 Saint Herblain
*E-mail address:* delphine.garnier@univ-nantes.fr (D. Garnier).

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ATP6V1G1 siRNA experiment, compensation of the inhibition of a V-ATPase by other ones could be one of the obstacles to overcome before we can imagine the blocking of ATP6V1G1 as a way to prevent the LOs-mediated transformation of recipient cells. The targeting of vesiculation itself could also offer an interesting alternative to block the oncogenic role of V-ATPases without affecting their physiological function. Future investigation and testing in *in vivo* models will therefore be needed before to determine if V-ATPase inhibition could be efficient at treating GBM, but these data so far offer great promise by the novelty of the approach, and will hopefully lead to new development for the treatment of GBM.

## Conflict of interest

The author declares no conflicts of interest.

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