Commentary

Glioblastoma stem cells and the importance of endolysosomes to keep them in the niches

Bernard Rogister[1,2]

[1]Laboratory of Nervous System Disorders and Therapy, GIGA-Neurosciences Research Center, University of Liège, Liège, Belgium; [2]Department of Neurology, CHU and University of Liège, Liège, Belgium

Correspondence to: Bernard Rogister, MD, PhD. GIGA-Neurosciences, Avenue Hippocrate, 15, 4000 Liège, Belgium. Email: Bernard.Rogister@ulg.ac.be.

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It's not necessary to stress out the bad outcome of patients with glioblastoma (GBM): after the classical Stupp's protocol (large surgical resection, radiotherapy and chemotherapy using temozolomide), the general survival rate is globally 9% 2 years after diagnosis (1). This situation is mainly the consequence of a systematic tumor recurrence. Although not yet fully experimentally demonstrated, it is more and more accepted that these recurrences could be due to a subpopulation of GBM cells, the glioblastoma stem cells (GSCs) (2). At least, those GSCs have been demonstrated to play a role in GBM initiation and therapeutic resistance. Indeed, they are regarded as "stem cells" because they express stem cells markers, can differentiate into various cell types and are able to self-renew. Self-renewal is more restrictive than cell proliferation as it implies that at least one daughter cell is roughly identical to the mother cell, including the ability to perform the same number of cell cycles (3).

However, to self-renew, a normal somatic stem cell should be present in the niches, which could be functionally, rather than histologically, defined as a suitable cellular and molecular environment needed to maintain those stem cells quiescent most of the time, when development is done. Sometimes, those normal somatic cells undergo a cell cycle with an asymmetric division allowing them to give rise to a new stem cell and a progenitor cell. This last escapes the niche and actively undergoes cell cycles before differentiation (4). In the adult brain, including human, such niches hosting neural stem cells (NSC) have been so far located in the subventricular zones (SVZ) and in the *dentatus gyrus* (DG) of the hippocampus (5).

The cancer stem cells seem to be able to self-renew outside a normal somatic stem cells niche. In GBM in peculiar, although those GSCs exhibit a specific attraction to the SVZ (6,7), some of them seem able to create their own niches outside the SVZ or the DG. Those "homemade" GSC niches are hypoxic and perivascular (8), with a higher capillary permeability as it is described in SVZ for the normal stem cells (9). However, as GBM is a highly invasive tumor, the question of how invading GSC maintain their stemness, including their self-renewal property, outside their homemade niches remains open and was addressed in a recent study (10).

Shingu *et al*. used an *in vivo* GBM model in which they invalidate three tumor suppressor genes expression specifically in adult NSC when located in the SVZ: *Trp53*, *Pten* and *Qk*. If *Trp53* and *Pten* are known to be frequently invalidated by mutations in GBM (11), *Qk* is less known (12). *Qk* encodes QKI, a star-family RNA-binding protein involved in various aspects of RNA biology: RNA stability, RNA splicing, translation and miRNA processing. In GBM, it has been suspected to stimulate the differentiation of cancer cells (13).

First, Shingu *et al*. show that invalidation of *Qk* in NSC is followed by a higher number of BrdU-positive cells and NSC cell number. But *in vitro*, it appears that the *Qk* invalidation is responsible of a slow-down of the cell cycle with a higher degree of self-renewal in NSC, even in presence of low concentrations of both EGF and bFGF, the two classical growth factors for these cells. Meanwhile, both *in vitro* and *in vivo*, *Qk* invalidation decreases the cell differentiation into

neurons, astrocytes and oligodendrocytes.

Then, Shingu *et al*. compared the consequences of invalidation of *Pten* and *Trp53* with or without a *Qk* invalidation in NSC. The invalidation of both *Pten* and *Trp53* in NSC is followed by an enlargement of the SVZ due to a higher number of NSC but when those NSC leave the SVZ, they stop to self-renew and differentiate into neurons, astrocytes and oligodendrocytes. Moreover, in those *Pten*$^{-/-}$ and *Trp53*$^{-/-}$ NSC mice, no glioma arise during the first 12 months of life. But when Shingu *et al*. look at the triple *Pten*$^{-/-}$, *Trp53*$^{-/-}$ and Qk$^{-/-}$ NSC mice, they could observe NSC outside the SVZ: in thalamus, in hypothalamus, in striatum or in the cortex, they found cells that, after undergoing the recombination, do not expressed differentiation markers but still express NSC markers (Ki67, Sox2). Once again, the absence of *Qk* expression in NSC decreased the proliferation rate of NSC, even in absence of PTEN and p53 proteins, but increases the self-renewal ability in these cells with a lower dependence to growth factors *in vitro*.

But the breakthrough of this paper is the fact that 92% of mice invalidated for those three genes in NSC develop a glioma with a median survival time of 105 days. Nearly half of these gliomas arise in the cortex, the others from various parts in the central nervous system. Moreover, they could be classified as GBM as these tumors are invasive and exhibit necrosis and hypervascularity with neoangiogenesis. On the basis of transcriptomic profiling, those GBM represented all four types (proneural, neural, classical and mesenchymal).

The next question addressed by Shingu *et al*. concerns the role of QKI in NSC and how its absence can promote glioblastomagenesis. As QKI has been shown to regulate various aspects of RNA biology (12), they performed a transcriptomic and a proteomic comparison of NSC expressing or not *Qk*, with or without *Pten* and *Trp53* expression. Shingu *et al*. found 217 genes that were alternatively spliced in absence of QKI but also 290 up-regulated and 343 down-regulated proteins. Then, they performed a photoactivable-ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) in order to identify genes whose expression is directly modified by the QKI absence. By combining transcriptomic, proteomic and PAR-CLIP data, they identified 104 genes whose expression is changed, 73 genes that are alternatively spliced and 148 proteins whose level is modified in absence of QKI. The *in-silico* analysis of these modifications indicates that 15 molecular pathways are concerned by the absence of QKI and 12 of them are related to receptor signaling and trafficking. More precisely, about 40% of genes down-regulated at the mRNA, at the protein level or at the splicing by the absence of QKI are involved in endocytosis-mediated receptor degradation including various components of endosomes and lysosomes.

This observation coupled to the fact that in absence of QKI, NSC self-renew *in vitro* at a low growth factors concentrations, raised the hypothesis that *Qk* deletion maintains the self-renewal of stem cells outside the SVZ by down-regulating endolysosome-dependent degradation of growth factors receptors. In order to demonstrate this hypothesis, Shingu *et al*. demonstrate that in absence of QKI, there is a decrease of endolysosome levels in genetically modified-NSC but also in human GBM samples as various key components of this organelle appear to be down-regulated in TCGA data sets. Moreover, knocking-down *Lamp1* (a key component of lysosome during autophagy) in NSC invalidated for *Pten* and *Trp53* allows the development of tumors. On the contrary, expression of *Tfe3* (a key component needed for endolysosome biogenesis) in NSC invalidated for *Pten*, *Trp53* and *Qk* decreases their self-renewal ability. The receptors and/or growth factors which are present at a higher levels in *Qk*$^{-/-}$ NSC plasmatic membrane are Notch, Frizzled, Wnt5a and Wnt5b but also EGFR, a receptor whose gene is frequently amplified and/or mutated in GBM (14).

This study is important by several aspects: (I) it describes a new *in vivo* experimental model of GBM; (II) it stress out the importance for a normal stem cell to be in a niche to be able to self-renew; (III) it also strengthens the possibility that GBM originate from NSC, as this point is still a matter of debate. However some points remain unsolved. Indeed, Shingu *et al*. emphasize that a decrease of endolysosome induced by the absence of QKI allows the NSC to be able to self-renew outside the SVZ, a situation that could pave the way to a GBM transformation. However, they do not demonstrate why a NSC outside the SVZ becomes a tumor cell or, in other words, why NSC able to self-renew in the SVZ do not undergo a tumor transformation most of the time. This study thus suggests that a cell (I) that is able to self-renew; but (II) that self-renews in the wrong place (outside the niche) will probably experience a tumor transformation. Therefore, if we do consider the role of the SVZ in self-renewal of NSC, we thus have also to take into account the role of SVZ in order to prevent their tumor transformation. This could be obtained in SVZ by keeping a relative quiescence of NSC after the development or by the modulation of symmetric or asymmetric cell division (15). Indeed, this process is clearly dependent about the orientation

of the cleavage plane of the mother cell regarding to the presence of the ependymal wall in the SVZ. This orientation is obviously lost when NSC self-renew outside the SVZ. The next questions are thus: how a stem cell, outside the niche but still able to self-renew, becomes a cancer stem cell? Is the loss of the cleavage plane ruling the asymmetrical or symmetrical division responsible of such a transformation?

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