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Commentary

Release the ballast: Glioblastoma rises above radiation therapy by exporting miR-603 in extracellular vesicles to become treatment-resistant

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Glioblastoma (GBM) remains an incurable disease with a median survival of ~15-18 months post-diagnosis, despite ongoing efforts to improve treatment outcome [1]. Standard-of-care consists of maximum safe surgical resection followed by systemic temozolomide (TMZ) and local radiation. After initial treatment with this regimen, GBM universally recurs and subsequent lines of therapy have limited efficacy. As a result, there is a great need to understand the biology of acquired therapeutic resistance in this disease. One mechanism that has been proposed to confer GBM treatment unresponsiveness and recurrence is enrichment of cancer stem cells (CSCs), a population that is resistant to standard-of-care therapies and has the capacity to give rise to the recurrent tumor. In this issue, Ramakrishnan et al. report that GBM acquires a treatment-resistant CSC phenotype following radiation therapy by actively exporting miR-603 in extracellular vesicles (EV) [2].

Ramakrishnan et al. performed microRNA sequencing on paired patient GBM specimens obtained at initial diagnosis and recurrence, in order to identify microRNAs (miRNA/miR) potentially involved in therapeutic resistance. Through this unbiased approach they discovered that miR-603 was significantly lower in recurrent GBM specimens. A similar trend is observed in human GBM cell lines and freshly resected GBM tissue following irradiation, which was accompanied by an increase in the abundance of insulin growth factor 1 (IGF1), IGF1 receptor (IGF1R), and O6-methylguanine–DNA methyltransferase (MGMT), which are predicted targets of miR-603. The authors further established that loss of miR-603 led to a CSC phenotype of GBM cells, accompanied by therapeutic resistance to radiation and TMZ of cultured human GBM cell lines and mouse xenograft models. Acquired resistance to radiation due to loss of miR-603 was primarily mediated by de-repression of IGF1-IGF1R, while acquired resistance to TMZ was linked to de-repression of MGMT, a clinically used biomarker of TMZ response in GBM [3]. Accordingly, overexpression of miR-603 in an intracranial xenograft GBM model rescued the impaired radiation response and further synergized with radiation and TMZ combination therapy.

This study went on to reveal that the decreased levels of miR-603 driving post-radiation therapeutic resistance of GBM was a result of en masse export of this miRNA in EV (summarized in Fig. 1). Earlier studies established that GBM cells secrete EV containing RNAs and proteins, which serve as communication vectors to transfer protumorigenic signals to different cell types within the tumor microenvironment [4]. Since then, there has been great interest in interrogating EV-associated macromolecules, including miRNAs, in GBM pathogenesis, diagnosis, and treatment, albeit primarily focusing on the role of EV delivery to target cells. For example, transfer of specific miRNAs from mesenchymal stem cells in stroma via EV were shown to have a CSC-promoting or -restrictive role [5,6]. Ramakrishnan et al. assessed miRNA-mediated CSC regulation from a different angle, focusing on the EV-mediated export of a CSC-limiting miRNA as resistance mechanism. Still, their observation that miR-603 containing EV are taken up by microglia, the brain-resident innate immune cells, also opens the possibility of miR-603 delivery to non-tumor cells leading to effects beyond the therapeutic resistance observed in the microRNA-secreting GBM cells. Although, the functional consequence of this uptake remains to be elucidated, a recent study by Abels et al. demonstrated that transfer of another miRNA, miR-21, can alter the proliferation status of GBM-associated microglia [7]. These observations support further investigation of CSC-independent roles of EV-associated miR-603 export in therapeutic resistance and its potential role in the repolarization of microglia into a tumor-supportive phenotype.

Building on the authors' findings, reversal of therapeutic resistance by augmenting miR-603 is a strategy that warrants investigation in GBM, and perhaps even other tumors. To this point, systemic delivery of miR-603 containing liposomes significantly delayed the growth of the triple-negative breast tumors in mice by targeting cellular proliferation and migration [8]. Regarding targeting of the downstream IGF1-IGF1R axis, previous studies in preclinical models showed that IGF1 blockade primes radiation response and reduces GBM tumor burden

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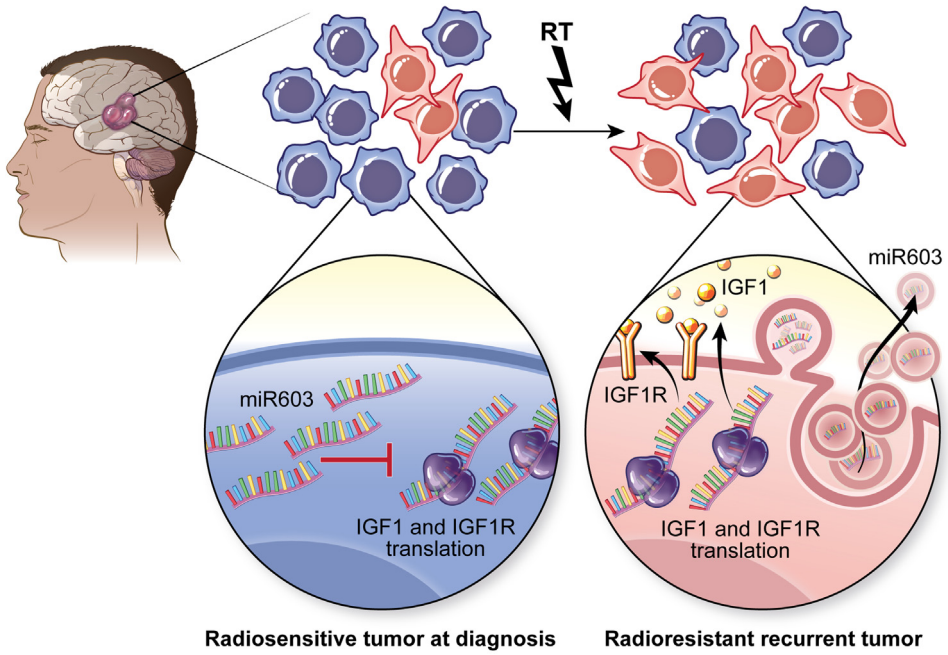

Fig. 1. miR603 suppresses IGF1 and IGF1R translation in radiosensitive, non-cancer stem cells (blue cells) at GBM diagnosis. After radiation therapy (RT), miR603 is exported via extracellular vesicles, resulting in expression of IGF1 and IGF1R, as well as a radioresistant, cancer stem cell phenotype (red cells). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[9]. However, clinical studies of IGF1R blockade in various cancers proved ineffective [10]. Ramakrishnan et al. describe a treatment-resistant phenotype driven by the export of miR-603, and resulting in de-repression of multiple downstream resistance genes, including TMZ resistance through concomitant regulation of MGMT expression. These observations provide a possible explanation as to why targeting IGF1 or IGF1R alone is insufficient to modulate the full spectrum of post-radiation resistance. Therefore, this study suggests that additional therapeutic targets could be identified by further exploring potential triggers of the novel mechanism of therapeutic resistance described, and by elucidating the upstream factors and signaling pathways leading to the packaging and expulsion of miR-603 EV.

Declaration of Competing Interest

The authors declare no conflict of interest.

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