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REVIEW

MicroRNA-7: a critical sensitizer for TRAIL sensitivity in glioblastoma cells

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TRAIL (TNF-related apoptosis-inducing ligand) is a potential anticancer agent because of its tumor-specifc apoptosis inducer activity without scathing normal cells. MicroRNAs (miRNAs) emerge as important regulators of cell viability. Our recent studies showed that miR-7 is a potential sensitizer for TRAIL-induced apoptosis in glioblastoma (GBM) cells, and XIAP is a critical gene in the apoptotic process as a direct downstream gene of miR-7. Additionally, this regulatory axis could also exert in other types of tumor cells. More importantly, we confirmed that co-delivery of sTRAIL and tumor suppressor miR-7 by MSCs leads to synergistic cancer killing effect. Thus, miR-7 has been demonstrated to be a critical sensitizer for TRAIL-induced apoptosis through regulating XIAP and highlights a novel therapeutic strategy for the treatment of GBM.

Keywords: glioblastoma; microRNA; TRAIL; mesenchymal stem cells, MSCs; exosome

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Introduction

Glioblastoma multiforme (GBM) is still one of the most lethal forms of brain tumor despite of the improvements in treatments. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) gained much attention during the past decade due to its therapeutic potential as a tumor-specific apoptosis inducer without affecting normal cells [1, 2]. However, the clinical use of recombinant TRAIL, have been hampered by its short half-life, unstable property and endogenic resistance to apoptosis *in vivo* [3].

Recently, an increasing number of studies have demonstrated that directly targeting primary tumor masses or even metastatic lesions by genetically modified mesenchymal stem cells (MSCs) with therapeutic agents could be a promising therapeutic approach [4]. Therefore, one strategy to overcome this challenge is to genetically modify TRAIL in MSCs. MSCs have been demonstrated to be a promising vehicle for delivering therapeutic agents to tumor tissues because of its tumor tropism [5]. It has partially counteracted the shortcoming of TRAIL treatment [6]. Meanwhile, Current clinical trials using systemic

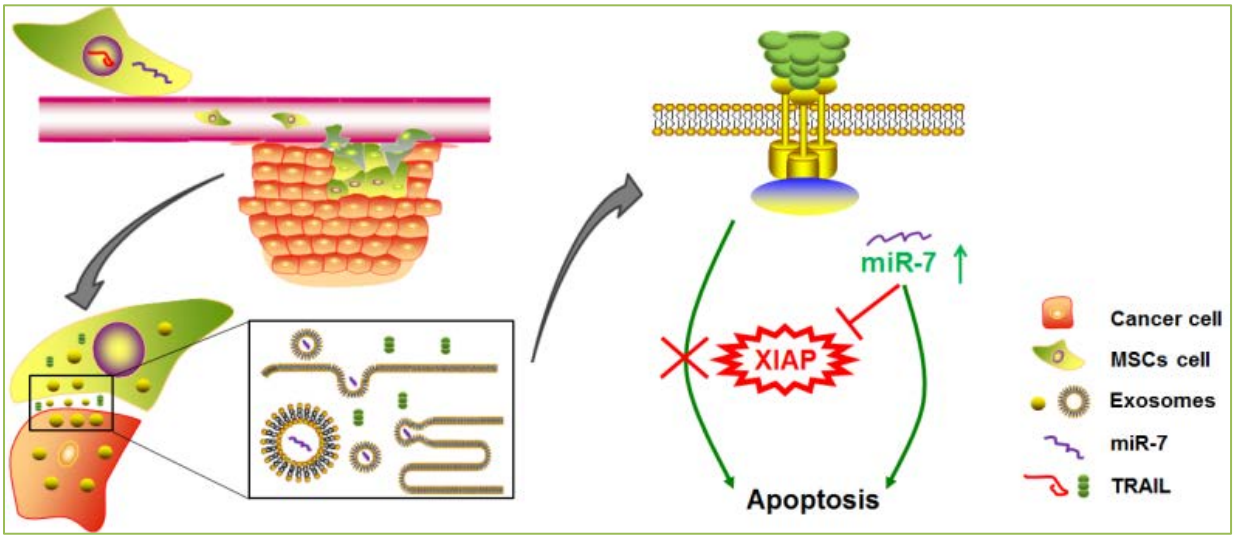

Figure 1. Synergistic effect of TRAIL and miR-7 co-expressing MSCs for cancer killing. TRAIL-expressing MSCs were established by lentiviral expression system. The cells were transiently transfected with miR-7 mimics. TRAIL/miR-7 co-expressing MSCs were intravenously injected into tumor-burdened animals. They were recruited to local tumor. TRAIL is secreted and induces cancer cell apoptosis. MiR-7 transfers to cancer cells from MSCs via exosomes and enhances TRAIL sensitivity by reducing XIAP expression in tumor tissues.

administration of TRAIL or TRAIL receptor sensitizers have largely been unsuccessful revealed the importance of TRAIL resistance, even when these targeted drugs are known to penetrate into tumor tissues [7-12]. This indicates that some inherent defects in the apoptotic program limit its utilization in the clinical application [13]. The defects may contribute to drug resistance and tumor progression and may be caused by deregulated expression of anti-apoptotic molecules.

MicroRNAs (miRNAs) are evolutionarily well-conserved, small non-coding transcripts. Accumulating evidences demonstrated that miRNAs are emerging as key regulators of multiple pathways involved in cancer development and progression [14]. In recent years, a serial of miRNAs has been demonstrated to play important roles in negatively regulating TRAIL sensitivity in GBM cells [15, 16]. They impair TRAIL-dependent apoptosis by inhibiting the expression of key functional proteins. For example, miR-21 suppresses TRAIL sensitivity in GBM cells by targeting a p53 family member TAp63. And miR-30 inhibits TRAIL responsiveness through inhibition of caspase-3 activation in GBM cells [16].

However, even nowadays, there is no systematic screening for miRNA-based natural sensitizers for TRAIL sensitivity in GBM cells. In the current study, using a global analysis in TRAIL sensitive and resistant GBM cells, we identified that miR-7, a tumor suppressor microRNA could be a potential sensitizer for TRAIL sensitivity in GBM cells. Our gain and loss of function experiments validated that overexpression of miR-7 increases TRAIL sensitivity in resistant GBM cells, whereas miR-7 inhibition significantly decreases responsiveness of GBM cells to TRAIL treatment. Moreover, we also provided evidences that this function is not restricted to GBM, but also present in other tumor types such as hepatocellular carcinoma (HCC) cells. As a brain enriched miRNA, we suppose that miR-7 could be a multifaceted molecular regulating multiple signaling pathways under physiological and pathological situations. Here, we demonstrated that miR-7 is a natural sensitizer for TRAIL sensitivity in GBM cells.

When we wondered to know the detailed molecular mechanism of miR-7's effect on TRAIL sensitivity in GBM cells, we found that an IAP family member XIAP is a direct target gene of miR-7. XIAP blocks apoptosis downstream of mitochondria by binding to and inhibiting caspase-3 and caspase-9 [17, 18]. An increasing number of clinical trials of XIAP targeted cancer treatment further confirmed that XIAP is a node molecule regulating cell death sensitivity [19, 20]. Moreover, the results of our gene silencing and rescue experiments support the opinion that miR-7-XIAP axis contributes to TRAIL sensitivity in GBM and other tumor types.

In recent years, a growing number of evidences demonstrated that exosomes could be a naturally delivery tool to transfer miRNAs for cell-cell communication [21-23]. All reports support that cells can secrete miRNAs and deliver them into recipient cells where the exogenous miRNAs can regulate target gene expression and recipient cell function. In our study, we speculated that enforced expression of exogenous miR-7 in sTRAIL-overexpressed mesenchymal stem cells may increase apoptosis and suppressed tumor growth in an exosome dependent manner. Finally, our

proof-of-principle study showed that therapeutic miR-7 produced in MSCs and loaded into extracellular exosomes could lead to synergistic antitumor efficacy with sTRAIL by inhibiting its target gene XIAP.

In summary, we screened and identified that miR-7 is a *bona fide* sensitizer for TRAIL sensitivity in GBM and also other tumor types. The mechanistic study showed that miR-7-XIAP axis plays a critical role for TRAIL sensitivity in cancers. As shown in Figure 1, we sought to evaluate the combined effect of exosome-transferred miR-7 and MSCs-mediated soluble TRAIL (sTRAIL) delivery on tumor growth *in vitro* and *in vivo*. We confirmed here that combining miR-7 overexpression with sTRAIL leads to synergistic tumor suppression effect *in vitro* and *in vivo*. Our study provides evidence for the co-delivery of sTRAIL and tumor suppressor miRNAs by MSCs to tumor tissues via exosomes and may highlight a novel therapeutic strategy for GBM.

Conflicting interests

The authors have declared that no conflict of interests exist.

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