Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MiR-216a-5p/Hexokinase 2 axis regulates uveal melanoma growth through modulation of Warburg effect

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ARTICLE INFO

Article history:
Received 8 May 2018
Accepted 11 May 2018
Available online xxx

Keywords:
MiR-216a-5p
HK2
Uveal melanoma
Warburg effect

ABSTRACT

Hexokinase-2 (HK2), the initial as well as the rate-limiting step in glycolysis, is overexpressed in many human cancers, and correlates with poor clinical outcomes. Aerobic glycolysis is a hallmark of cancer, and drugs targeting its enzymes, including HK2, are being developed. However, the mechanisms of HK2 inhibition and the physiological significance of the HK2 inhibitors in cancer cells are rarely reported. Here, we show that microRNA-216a-5p (miR-216a-5p) inhibits HK2 expression by directly targeting its 3′-UTR in uveal melanoma cells. Through inhibition of HK2, miR-216a-5p dampens glycolysis by reducing HK activity, glucose uptake, lactate production, ATP generation, extracellular acidification rate (ECAR), and increasing oxygen consumption rate (OCR) in uveal melanoma cells. Importantly, glycolysis regulated by miR-216a-5p is critical for its regulating uveal melanoma tumor growth both in vitro and in vivo. miR-216a-5p expression is negatively correlated with HK2 expression and predicts better outcome in uveal melanoma patients. Our findings provide clues regarding the role of miR-216a-5p as a tumor suppressor in uveal melanoma through the inhibition of HK2. Targeting HK2 through miR-216a-5p could be a promising therapeutic strategy in uveal melanoma.

Uveal melanoma is one of the most common primary intraocular malignant cancers with a high death rate of about 50% [1–4]. Nowadays, no effective treatment is available for patients suffering from uveal melanoma owing to the poorly characterized mechanisms of the uveal melanoma initiation [5–7]. Despite several advances in surgery, chemotherapy, and radiotherapy, the five-year of survival rate is still unsatisfied [8–11]. Thus, it is crucial to study the molecular mechanisms of uveal melanoma growth and to search for the novel therapeutic targets of uveal melanoma.

Aerobic glycolysis, which displays abnormal metabolism characterized by high glycolysis even in the presence of abundant oxygen, is acknowledged as a common feature of cancer cells [12,13]. This phenomenon accelerates tumor growth with increased glucose uptake and lactate production. Therefore, understanding of how to harness this process is important to identify promising targets for cancer treatment.

Hexokinase 2 (HK2), the initial as well as the rate-limiting step in glycolysis, which phosphorylates glucose to generate glucose-6-phosphate, is an insulin-inducible isoform mainly found in insulin-sensitive tissues such as adipose tissues and skeletal muscles [14–16]. HK2 is highly expressed in several cancers, such as breast cancer [17], lung carcinoma [18], hepatocellular carcinoma [19], ovarian cancer [20], gastric cancer [21], etc., and helps drive tumor growth by maintaining high glycolysis rates of rapidly growing tumors. In particular, higher expression of HK2 is associated with more advanced clinical stage and poorer progression-free survival.

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https://doi.org/10.1016/j.bbrc.2018.05.069
0006-291X/

HK2 expression can be stimulated by multiple factors, such as hypoxia inducible factor 1 α (HIF-1α) [22], p65 [23], etc. Patra KC et al. reported HK2 ablation inhibits the neoplastic phenotype of human lung and breast cancer cells in vitro and in vivo [24]. However, the mechanisms of HK2 inhibition and the physiological significance of the HK2 inhibitors in cancer cells still need further investigation.

miRNAs have been reported to play critical roles in many cell process, such as cell growth, differentiation, and apoptosis [25,26]. Dysregulation of miRNAs is responsible for the varieties of pathogenesis of cancer, and several miRNAs may be served as potential important diagnostic and therapeutic agents in human cancers [27,28]. In our previous study, we identified the suppressive role of miR-216a-5p in osteosarcoma cancer cells [29]. However, whether miR-216a-5p regulates glucose metabolism is unknown.

In the present study, we demonstrate that miR-216a-5p inhibits glycolysis in uveal melanoma cancer cells, leading to the inhibition of cancer cell proliferation both in vitro and in vivo. Mechanistically, HK2 is a novel target of miR-216a-5p. By targeting HK2 directly, miR-216a-5p inhibits uveal melanoma cancer cell glycolysis and growth. In uveal melanoma patients, miR-216a-5p abundance is negatively correlated with HK2 expression and positively correlated with the better survival.

1. Results

1.1. Aerobic glycolysis is involved in the regulation of uveal melanoma cell proliferation by miR-216a-5p

Our previous work demonstrated the tumor suppressive role of miR-216a-5p, and since aerobic glycolysis plays a critical role in cancer proliferation, we investigated if aerobic glycolysis plays a role in the inhibition of uveal melanoma cell proliferation mediated by miR-216a-5p. Anti-miR-216a-5p to promotes proliferation in A375 and MUM-2B cells, and more importantly, the glycolytic inhibitor 2-deoxy-D-glucose (2-DG) suppressed the ability of anti-miR-216a-5p to promote proliferation in A375 and MUM-2B cells (Fig. 1A and B), indicating that aerobic glycolysis is critical in the regulation of cell proliferation by miR-216a-5p.

1.2. HK2 is identified as a direct target of miR-216a-5p

To further study the relationship between miR-216a-5p and aerobic glycolysis, we searched for the potential targets of miR-216a-5p in publicly available databases (TargetScan and miRanda). Multiple genes were predicted as the potential target genes of miR-216a-5p, from which we chose those reported to play roles in glycolysis. Next, we performed Western blot analysis to validate the potential targets in human kidney embryonic HEK293T cells. Consistent with our previously reported result [28], overexpression of miR-216a-5p suppressed the CDK14 oncogene expression (Supplementary Fig. S1). Moreover, miR-216a-5p repressed the HK2 expression, a key enzyme of glycolysis, but not the expression of another key enzyme of glycolysis, PKM2. As a result, we picked out HK2 for further study.

As expected, transfection of miR-216a-5p mimics suppressed HK2 expression in A375 and MUM-2B cells (Fig. 2A). In contrast, miR-216a-5p inhibitor increased those of HK2 expression (Fig. 2B). To further examine how miR-216a-5p influenced the expression of HK2, we detected the HK2 mRNA level after transfection of either miR-216a-5p mimics or miR-216a-5p inhibitor into A375 and MUM-2B cells. The level of HK2 mRNA was down-regulated upon miR-216a-5p overexpression, whereas up-regulated upon miR-216a-5p inhibition (Fig. 2C).

Next, to determine whether the negative regulatory effects of miR-216a-5p on HK2 expression were caused by direct binding of HK2, we transfected A375 and MUM-2B cells with wild-type HK2 3′-UTR (3′-untranslated region) or mutated HK2 3′-UTR luciferase reporter and miR-216a-5p. miR-216a-5p reduced the wild-type HK2 3′-UTR reporter activity, but not the reporter luciferase activity in which the binding sites for miR-216a-5p were mutated (Fig. 2D). Collectively, these results suggest that miR-216a-5p inhibits HK2 expression by directly targeting its 3′-UTR in uveal melanoma cells.

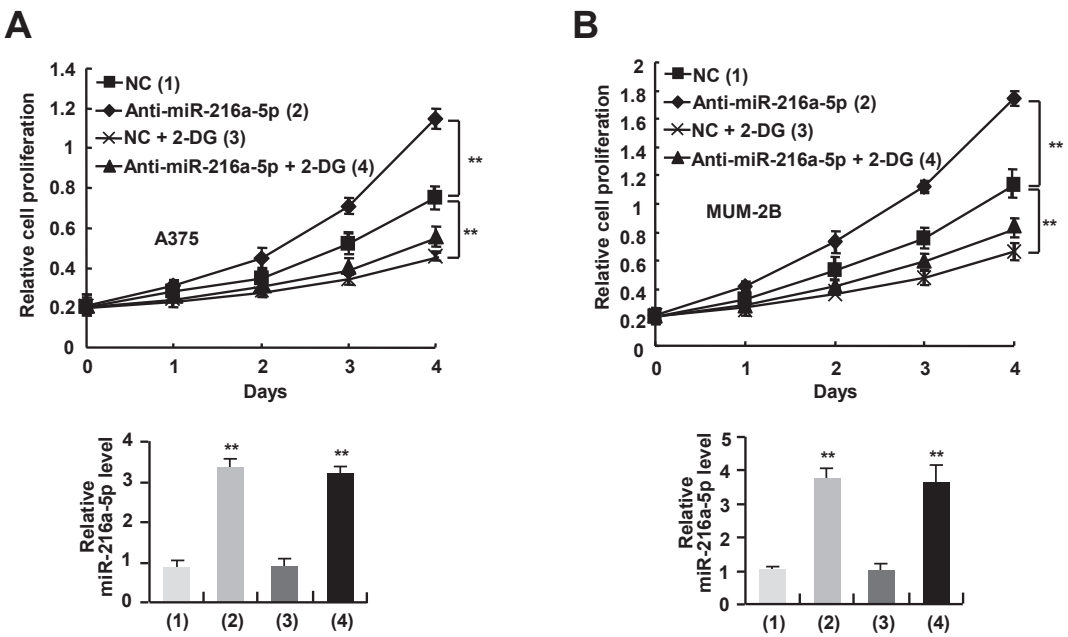

Fig. 1. Aerobic glycolysis is involved in the regulation of uveal melanoma cell proliferation by miR-216a-5p. The proliferation curve of A375 (A) and MUM-2B (B) uveal melanoma cells transfected with anti-miR-216a-5p or non-specific control for miRNA (NC) and treated with 2.5 mM 2-DG as indicated. Quantitative real-time PCR (qRT-PCR) analysis shows miR-216a-5p expression. Data shown are mean ± SD of triplicate measurements that have been repeated three times with similar results. $^{**}P < 0.01$.

Please cite this article in press as: Y. Liu, et al., MiR-216a-5p/Hexokinase 2 axis regulates uveal melanoma growth through modulation of Warburg effect, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.069