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REVIEW

Reciprocal regulation among miR-181d/CRY2/FBXL3/c-myc signaling axis modulates metabolism in colorectal cancer

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Patients with CRC (colorectal cancer) usually have a poor prognosis and the cure rate of CRC remained unsatisfied due to unfavorable curative effect. It is well known that microRNAs (miRNAs) and energy metabolism have pivotal roles in CRC progression. In a recent article in Cell Death & Disease by Xiaofeng Guo. *et al*. 2017, we have reported an oncogenic role of miR-181d in CRC by promoting glycolysis, and its underlying molecular mechanism about a new feedback loop among miR-181d/CRY2/FBXL3/c-myc signaling axis. Among these, we have identified the level of miR-181d was upregulated in CRC and the inhibition of miR-181d decreased glycolysis in CRC cells. We also found that c-myc played a central role in regulating cell glycolysis, which is required for the metabolic shift induced by miR-181d. Besides, we have demonstrated FBXL3 and CRY2 were direct targets of miR-181d and c-myc promoted miR-181d upregulation while inhibiting the expression of CRY2 and FBXL3 in CRC cells. The data from our recent article strongly suggest a new light onto the oncogenic function of the miR-181d in CRC. Furthermore, these findings represent a novel potential approach for silencing miR-181d/c-myc signaling pathway in CRC treatment.

Keywords: Metabolism; miR-181d; c-myc; colorectal cancer

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Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer death in the world with characteristics of fast progression, unfavorable curative effect, and poor prognosis [1]. According to newly published "Cancer Statistics, 2017" of United States, the estimated new CRC cases were 135,430, and the new deaths were 50,260 in 2017 [2]. In the past two decades, significant advances have been made in our understanding of CRC. Nonetheless, the cure rate of CRC remains insufficient and approximately 30%–50% of patients who undergo curative resection subsequently experience local and systemic recurrence, and the prognosis of patients with liver metastasis is still poor [3, 4]. Therefore, it was urged to make further exploration and search new targeted therapeutic approaches to get its morbidity and mortality under control [5].

In the past few decades, investigators have devoted considerable efforts to explore the etiology and pathogenesis of CRC [6]. CRC has variable genetic signatures, which can be as unique as the individual host, and it develops through at least 3 major pathways, which include chromosomal instability, mismatch repair, and methylate phenotype [7].

However, the majority of recent studies have shown that in cancer cells the alterations of glucose metabolism are associated with miRNA dysregulation [8, 9] Reprogrammed energy metabolism to fuel rapid cell growth and proliferation is an emerging hallmark of cancer. Altered glucose metabolism in cancer cells is termed as 'Warburg effect', which describes the propensity of most cancer cells to take up glucose avidly and convert it primarily to lactate, despite available oxygen [10, 11]. The Warburg effect has been observed in many tumor types [12]. Recently progress had been made regarding the mechanistic understanding of glucose metabolism and associated diagnostic and therapeutic methods, which have been investigated in colorectal cancer.

Large studies suggest that miRNA is involved in the development of CRC and plays important roles in multiple biological processes of CRC, such as cell growth, development, differentiation, survival, etc. [13-15]. Moreover, miRNAs have been shown to be involved in tumorigenesis where they function as tumor suppressors or oncomiRs. In addition, miRNAs participate in cell metabolism by regulating the expression of genes whose protein products either directly regulate metabolic machinery or indirectly modulate the expression of metabolic enzymes, serving as master regulators [16-18]. For example, miR-26a regulates glucose metabolism of CRC cells by directly targeting the pyruvate dehydrogenase complex component X (PDHX), which inhibits the conversion of pyruvate to acetyl coenzyme A in the citric acid cycle [19]. Furthermore, some miRNAs have been identified as diagnostic indicators, novel therapeutic targets in CRC. Therefore, identifying therapeutic miRNAs would be of great clinical value.

As here highlighted, we have recently reported for the first time that miR-181d served as a critical oncogene, which played an important role in the metabolism of CRC [5]. The miR-181family includes 4 members, miR-181a, miR-181b, miR-181c and miR-181d. MiR-181a and miR-181b are transcribed from two separated gene loci (miR-181a-1/miR-181b-1 and miR-181a-2/ miR-181b-2). MiR-181c and miR-181d are transcribed from another locus [20]. Interestingly, effects of miR-181 family members on tumors are totally divergent. For example, miR-181a/b was reported to be dysregulated, and acted as either an oncogene or a tumor-suppressor gene via affecting metabolic shift [21]. In our study we found that miR-181d was significantly upregulated in human CRC tissues, cell lines and mouse models compared with their controls. In addition, our clinicopathological assays showed that miR-181d dysregulation was associated with CRC metastasis and TNM stage. Functionally, we found that high expression of miR-181d can promote cell proliferation, migration, and invasion, strongly suggesting that miR-181d was a prognostic indicator of CRC cell growth and metastasis and acts as an oncomiR in CRC. We also found that miR-181d was essential for CRC cell glycolysis. The inhibition of miR-181d by siRNA significantly decreased the level of the glycolysis under basal conditions, the glycolytic capacity, and the glycolytic reserve. In conclusion, Our study was the first to show that miR-181d exerted its oncogenic roles by promoting the Warburg effect.

It has also become clear that the multifaceted oncogene, c-myc, plays an important regulatory role in glucose metabolism [22, 23]. The c-myc protein functions as a helix-loop-helix transcription factor and is a key regulator of cell proliferation and cell fate decision [24]. To explore whether other factors are critical for the inhibition of miR-181d decreases glycolysis in CRC cells. We investigated the expression of c-myc, hypoxia inducible factors α (HIF1α), and PKM2 after miR-181d overexpression and showed that only c-myc increased, which strongly indicated that c-myc was required for the metabolic shift induced by miR-181d. On the other hand, anti-miR-181d, 10058-F4, or FX11, the inhibitor of miR-181d, c-myc and LDHA, significantly inhibited the process of glycolysis observed in miR-181d-overexpressing cells. Therefore, we suggested that upregulation of miR-181d in CRC promoted glycolysis, which may be mediated by c-myc and responsible for aggressiveness of CRC cells.

Although c-myc mediates the effect of miR-181d on glycolysis in CRC cells, c-myc is not a direct target of miR-181d. To further reveal the mechanism of miR-181d-induced glycolysis, we predicted potential direct target of miR-181d through three different algorithms. We identified CRY2 and FBXL3 as two direct targets of miR-181d based on the following evidence that (i) miR-181d overexpression significantly decreased CRY2 and FBXL3 protein expression; (ii) CRY2 and FBXL3 and miR-181d expression levels were negatively correlated; and (iii) 3′-UTR-luciferase reporter activities of both CRY2 and FBXL3 were suppressed by miR-181d overexpression. It is known from the literature that CRY2/FBXL3 is able to decrease the expression of c-myc via driving proteolytic turnover of T58-phosphorylated c-myc [25]. So, we concluded that miR-181d stabilized c-myc through downregulating CRY2/FBXL3.

Next, we sought to further investigate the transcriptional regulation of miR-181d. Though software analysis, luciferase reporter assay and CHIP assay, we proved that c-myc bound the promoter of miR-181d and augmented its expression. Thus miR-181d forms a positive feedback loop with c-myc.

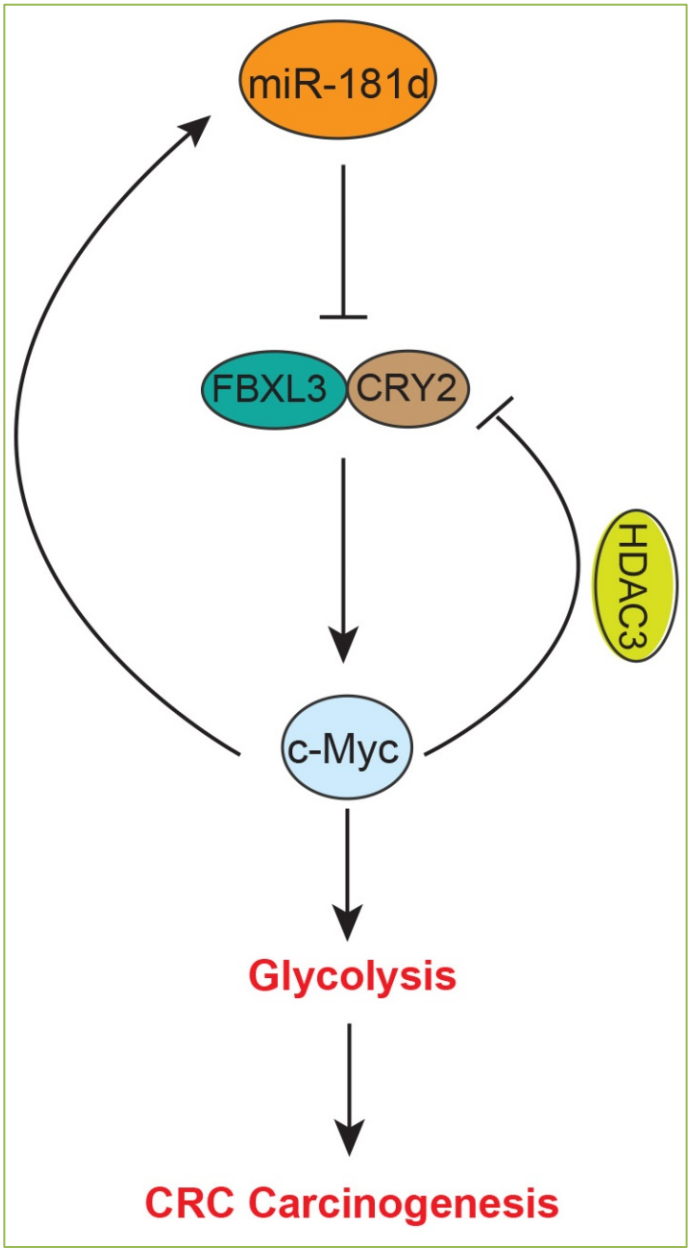

Figure 1. miR-181d mediates a novel feedback loop in CRY2/FBXL3/c-myc signaling and regulates glucose metabolism.

Interestingly, previous report showed that c-myc can form a complex with HDAC1 or HDAC3 and transcriptionally repress down-stream gene expression [26]. Thus, we performed a co-IP and demonstrated that c-myc and interacted with HDAC3 instead of HDAC1. Furthermore, CHIP-PCR revealed that c-myc/HDAC3 complex impaired the expression of CRY2/FBXL3 by binding their promoters, which indicated that c-myc can form a negative feedback with CRY2/FBXL3.

In summary, our recent publication demonstrated for the first time a miR-181d/CRY2/FBXL3/c-myc reciprocal feedback loop linking overexpression of miR-181d and c-myc with CRY2/FBXL3 suppression in CRC glucose metabolism (Figure 1), thus emphasizing the oncogenic role of miR-181d in CRC. Our present findings represent a novel potential approach for silencing c-myc/miR-181d signaling pathway in CRC treatment.

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Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

miR-181d: microRNA-181d; CRC: Colorectal cancer; CRY2: Cryptochrome2; FBXL3: F-box and leucine rich repeat protein 3; LDHA: A lactate dehydrogenase; HIF1α: Hypoxia inducible factors α; PKM2: Pyruvate kinase M2.

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