Journal of Pediatric Surgery (2012) 47, 1797–1805

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Micro-RNA-21 regulates the sensitivity to cisplatin in human neuroblastoma cells

Yun Chen[a,b], Ya-Hui Tsai[a,b,*], Yu Fang[a], Sheng-Hong Tseng[c,*]

[a]*Department of Surgery, Far Eastern Memorial Hospital, Pan-Chiao, New Taipei, Taiwan*
[b]*Department of Chemical Engineering and Materials Science, Yuan Ze University, Chung-Li, Taoyuan, Taiwan*
[c]*Department of Surgery, National Taiwan University Hospital, and National Taiwan University College of Medicine, Taipei 100, Taiwan*

Received 15 December 2011; revised 10 April 2012; accepted 9 May 2012

Key words:
miR-21;
Cisplatin;
Neuroblastoma cells;
Drug resistance

Abstract

Background/purpose: Drug resistance often causes treatment failure in neuroblastomas. Increasing evidence has implicated that the micro-RNAs (miRNAs) are involved in the development of drug resistance. In this report, we aimed to investigate the role of miRNA in cisplatin resistance of neuroblastoma cells.

Materials and methods: The cell viability of the neuroblastoma cells after cisplatin treatment was analyzed. The expression of the miRNAs and phosphatase and tensin homolog (PTEN) messenger RNA in the neuroblastoma cells was studied by real-time polymerase chain reaction. Overexpression of miRNA or suppression of miRNA expression by antagomir was used to investigate the effects of miRNA on the cisplatin-induced cell death or proliferation.

Results: The expression of miR-21 was increased in the cisplatin-resistant (CisR) neuroblastoma cells as compared with the parental cells, and the antagomir against miR-21 converted the resistant cells into sensitive ones. Ectopic expression of pre-miR-21 in parental cells resulted in decreased sensitivity to cisplatin treatment. In addition, overexpression of pre-miR-21 markedly increa sed the proliferation rate of neuroblastoma cells. The level of PTEN messenger RNA and protein in the CisR cells was lower than that in the parental cells. Transfection of pre-miR-21 into the parental cells reduced the PTEN expression, and transfection of anti-miR-21 into the CisR cells increased the PTEN expression.

Conclusion: Micro-RNA-21 regulated the drug resistance and proliferation in neuroblastoma cells.

1. Background

Neuroblastoma is the most common extracranial solid malignancy in children [1,2]. It is an aggressive tumor arising from neuroblasts and usually begins in the nerve tissue of adrenal glands [1,2]. Neuroblastomas often are not detected until they have grown and compressed surrounding organs or have spread to the lymph nodes, bones, bone marrow, or

* Corresponding authors. Ya-Hui Tsai is to be contacted at Department of Surgery, Far Eastern Memorial Hospital, Banciao, Taipei 220, Taiwan. Tel.: +886 2 89667000x2923; fax: +886 2 89665567. Sheng-Hong Tseng, Department of Surgery, National Taiwan University Hospital, Taipei 100, Taiwan. Tel.: +886 2 23123456x65110; fax: +886 2 89665567.
E-mail addresses: yahuitsai@gmail.com (Y.-H. Tsai), tsh5110@ntu.edu.tw (S.-H. Tseng).

0022-3468/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jpedsurg.2012.05.013

central nervous system [1,2]. Currently, the standard treatment regimen for neuroblastomas includes surgery, radiotherapy, and chemotherapy [3]. However, the prognosis of patients with neuroblastomas is still poor, especially for stage 4 disease in children older than 1 year [1-3]. Approximately 70% to 80% of patients older than 18 months present with metastatic disease, usually in the lymph nodes, liver, bone, and bone marrow [2-4]. Less than half of these patients are cured, even using high-dose chemotherapy followed by autologous bone marrow or stem cell rescue [2-4].

The chemotherapeutics used for neuroblastomas include Vinca alkaloids, anthracyclines, epipodophyllotoxins, camptothecins, and others, all of which function by causing nucleotide damage or mitosis inhibition and the resultant cell cycle arrest and apoptosis [3,5,6]. However, drug resistance often happens to result in treatment failure, shown as tumor growth or tumor relapse [6,7]. The causes of drug resistance can be generally divided into 3 categories: (1) decreased uptake of drugs that require transporters to enter cells; (2) increased efflux of drugs that can easily diffuse into cell membrane; and (3) various intrinsic changes that diminish the capacity of cytotoxic drugs to kill cells, including reduced apoptosis, increased DNA repair, and altered metabolism of drugs [6-9]. Whether these causative mechanisms are owing to the specific nature of the cancer cell itself or the genetic and epigenetic changes that follow the treatment pressure has been under investigation in various types of cancer cells [6-9].

Micro-RNAs (miRNAs) are small RNAs with 19 to 23 nucleotides in length, which can be found in all mammalian cells. These miRNAs are incorporated into the RNA-induced silencing complex and then target the 3′-untranslated region of a specific messenger RNA (mRNA) by a seed sequence that is located near the 5′ region of the miRNA. The consequences of miRNA binding are that the mRNA is silenced or degraded, resulting in reduced expression level of the protein encoded by the targeted mRNA [10,11]. MicroRNAs have been found to regulate genes involved in various pathways such as cell death, cell proliferation, stress response, and metabolism. In recent years, the evidence of the involvement of miRNAs in the regulation of drug sensitivity/ resistance-related molecules has been emerging [10,11]. The expression profile of miRNA and their potential targets in cancer therapy was extensively explored in breast, lung, prostate, colon, gastric, and ovarian cancers [12-16]. In breast cancer cells, a relationship between increased tamoxifen resistance and reduced level of p27, and increased miR-221/222 expression had been revealed [17]. In addition, miR-451 was found involved in the regulation of multidrug resistance 1 (MDR1) expression in breast cancer cells [18]. In gastric cancer cells, miR-15b and miR-16 directly regulate the expression Bcl-2, thus modulating the susceptibility of cancer cells to anticancer drug-induced apoptosis [19]. The expression of miRNA-181b is significantly decreased in gastric and lung cancer cells with multidrug resistance, and this is also linked to the robust expression of Bcl-2 [20]. In ovarian cancer, a panel of miRNAs, let-7e, miR-30c, miR-125b, miR-130a, and miR-335, were found diversely expressed in resistant cell lines [21]. Further investigation revealed that Sirt1, an important molecule for keeping genome stability and cell survival, is the target of miR-34a [22]. There are also many reports presenting the regulation of transporter proteins (ABC family) and drug resistance by different miRNAs [23-26]. All the evidence suggests that miRNA regulation has become a critical epigenetic mechanism for the drug resistance or multidrug resistance formation. However, very few reports are concerned with the linking between miRNA and the chemosensitivity of neuroblastoma.

In this study, we attempted to find the critical miRNAs and their targets involving in the regulation of the drug resistance in neuroblastoma cells. Cisplatin was used in this project because it is commonly adopted for the treatment of neuroblastomas [3,5]. We induced cisplatin resistance in human neuroblastoma cells and investigated the miRNAs possibly involved in the mechanisms of the resistance to cisplatin in these cells.

2. Materials and methods

2.1. Cell lines and cell culture

Human SH-SY5Y and BE(2)-M17 neuroblastoma cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum plus 2 mM L-glutamine, 1 IU/mL penicillin G, and 1 μg/mL streptomycin. SH-SY5Y cell line is a cell line with single copy of MYCN, and BE(2)-M17 cell line is a MYCN-amplified cell line [27,28].

2.2. Selection of cisplatin-resistant neuroblastoma cell lines

To obtain neuroblastoma cells with cisplatin resistance, 2 neuroblastoma cell lines SH-SY5Y and BE(2)-M17 were treated with graded doses of cisplatin (Sigma-Aldrich Chemical Co, St Louis, MO) for 12 weeks. The doses of cisplatin were chosen according to the 50% inhibitory concentration (IC50, the drug concentration at which 50% of the cells were killed) of the neuroblastoma cells (IC50 of the SH-SY5Y cells to cisplatin for 24 hours was 39.4 μM and that of the BE(2)-M17 cells was 29.3 μM). During the selection process, each step included 2 days of treatment and 3 days of drug withdrawal and cycled with step-wised increment of cisplatin concentration. At the end of selection, we obtained the resistant cells that could survive in 25-μM cisplatin and maintain the resistance for at least 2 more months. The wild type cells were designated as parental cells and the cisplatin-resistant (CisR) cells were designated as CisR cells.