MOLECULAR ONCOLOGY 6 (2012) 48–61

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

CRABP-II methylation: A critical determinant of retinoic acid resistance of medulloblastoma cells

Yuan-Shan Fu[a,1], Qian Wang[a,1], Jing-Xin Ma[a], Xiang-Hong Yang[b], Mo-Li Wu[a], Kai-Li Zhang[a], Qing-You Kong[a], Xiao-Yan Chen[a], Yuan Sun[a], Nan-Nan Chen[a], Xiao-Hong Shu[a], Hong Li[a,*], Jia Liu[a,*]

[a]Liaoning Laboratory of Cancer Genomics and Department of Cell Biology, College of Basic Medical Sciences, Dalian Medical University, Dalian 116044, PR China

[b]Department of Pathology, Sheng-Jing Hospital, China Medical University, Shenyang 100023, PR China

ARTICLE INFO

Article history:
Received 17 October 2011
Received in revised form
14 November 2011
Accepted 14 November 2011
Available online 25 November 2011

Keywords:
Medulloblastoma
Retinoic acid
RA signaling
CRABP-II
DNA methylation
Epigenetic modulation: Gene transfection

ABSTRACT

Medulloblastoma cells exhibit varied responses to therapy by all-trans retinoic acid (RA). The underlying mechanism for such diverse effects however remains largely unclear. In this study, we attempted to elucidate the molecular basis of RA resistance through the study of RA signaling components in both RA-sensitive (Med-3) and RA-resistant (UW228-2 and UW228-3) medulloblastoma cells. The results revealed that RARα/β/γ and RXRα/β/γ were found in the three cell lines. Expression of CRABP-I and CRABP-II was seen in Med-3 cells, up-regulated when treated with RA, but was absent in UW228-2 and UW228-3 cells regardless of RA treatment. Bisulfite sequencing revealed 8 methylated CG sites at the promoter region of CRABP-II in UW228-2 and UW228-3 but not in Med-3 cells. Demethylation by 5-aza-2′-deoxycytidine recovered CRABP-II expression. Upon restoration of CRABP-II expression, both UW228-2 and UW228-3 cells responded to RA treatment by forming neuronal-like differentiation, synaptophysin expression, β-III tubulin upregulation, and apoptosis. Furthermore, CRABP-II specific siRNA reduced RA sensitivity in Med-3 cells. Tissue microarray-based immunohistochemical staining showed variable CRABP-II expression patterns among 104 medulloblastoma cases, ranging from negative (42.3%), partly positive (14.4%) to positive (43.3%). CRABP-II expression was positively correlated with synaptophysin (rs = 0.317; $p = 0.001$) but not with CRABP-I expression ($p > 0.05$). In conclusion, aberrant methylation in CRABP-II reduces the expression of CRABP-II that in turn confers RA resistance in medulloblastoma cells. Determination of CRABP-II expression or methylation status may enable a personalized RA therapy in patients with medulloblastomas and other types of cancers.

1. Introduction

Retinoic acid/RA has been commonly-used in cancer chemotherapy because it can induce differentiation and apoptosis (Freemantle, 2003). The anticancer signal of RA is transmitted from cytoplasm to the nucleus through stepwise processes. RA first binds to protein/CRABP-II and then transports lipophilic RA to the nucleus where RA regulates its target gene

* *Corresponding authors*. Tel./fax: +86 411 86110316.
E-mail addresses: lihongmcn@dlmedu.edu.cn, lihongmcn@yahoo.com.cn (H. Li), jialiudl@dlmedu.edu.cn (J. Liu).
[1] Equivalent authors.

1574-7891/$ – see front matter © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.molonc.2011.11.004

expression through binding with the heterodimers of its nuclear receptors, RARα/β/γ and RXRα/β/γ (Delva et al., 1999; Dong et al., 1999; Budhu and Noy, 2002; Sessler and Noy, 2005). Nevertheless, RA resistance often occurs at first-time use or during the treatment (Warrell et al., 1993; Freemantle et al., 2003). Although it is known that aberrant expression of nuclear RA receptors, increased rate of cytoplasmic RA metabolism, differential BMP-2 expression, disrupted expression of CRABP-II and the fatty acid binding protein/FABP-5-mediated RA signaling pathway may influence the RA sensitivity of cancer cells (van der Burg et al., 1993; Hallahan et al., 2003; Schug et al., 2007; Schug et al., 2008; Corlazzoli et al., 2009), the detailed mechanism(s) resulting in RA-resistance remains to be further elucidated. Since a normal operation of CRABP-II mediated RA signaling is essential for RA to exert anticancer effects, it is worthwhile to evaluate the status of this signaling in RA-sensitive and RA-resistant cancer cells with the same tissue origin.

Medulloblastoma is the most frequent primary brain malignancy in childhood and characterized with rapid growth, earlier intracranial dissemination and high recurrence incidence (Chatty and Earle, 1971; Ho et al., 2000; Fossati et al., 2009). Although the combination of operation with craniospinal radiation and/or multi-agent chemotherapy have been adapted in clinical settings (Mueller and Chang, 2009; Ohta et al., 2011), the outcome of medulloblastomas remains poor due to the difficulty in removing the highly invasive tumor radically and the long-term side effects of conventional adjuvant therapies (Gururangan et al., 2008). As a kind of neuroectodermal tumors, medulloblastomas maintain the potential for further differentiation. However, the medulloblastoma cells respond to RA differently by showing both differentiation and apoptosis (Gumireddy et al., 2003) or growth arrest only (Chang et al., 2007) or almost no response (Kitamura et al., 2004). According to our previous observations, human medulloblastoma cell line Med-3 is relatively sensitive to RA treatment (Liu et al., 2000), while UW228-1, UW228-2 and UW228-3 cells are not [unpublished data]. We therefore selected Med-3, UW228-2 and UW228-3 cells as an experimental model to investigate the underlying molecular mechanism for diverse sensitivities of medulloblastomas to the treatment by RA.

2. Materials and methods

2.1. Cell culture and treatment

The Med-3 medulloblastoma cell line was kindly provided by the doctors in the Department of Neurosurgery, Kobe University School of Medicine (Matsumoto, 1991). UW228-2 and UW228-3 cell lines were established and provided by the Department of Neurological Surgery, University of Washington at Seattle (Keles et al., 1995). Med-3 cells were cultured in MEM (Invitrogen, California, USA) containing 10% fetal bovine serum/FBS (Gibco Life Science, Grand Island, NY) and UW228-2 and UW228-3 cells in DMEM (Invitrogen) supplemented with 10% FBS. 5×10^4/ml of the cells were plated to 100 mm dishes (Nunc A/S, Roskilde, Denmark) and incubated for 24 h before further experiments. The gelatin-coated coverslips were put

Table 1 – Primer sequences for conventional RT-PCR and methylation-specific PCR.

Gene	Primers	Amplicon size (bp)	Annealing temperature (°C)
RARα	F:5′-CTGCCAGTACTGCCGACTGC-3′ R:5′-ACGTTGTTCTGAGCTGTTGTTCGTA-3′	325	66
β	F:5′-GAATTGAAACACAGAGCACC-3′ R:5′-GCAGGAGTGGTGACTGACTG-3′	1180	54
γ	F:5′-CCACCAATAAGGAGCGACTCTTTG-3′ R:5′-TTCTTCTGGATGCTTCGGCG-3′	358	55
RXRα	F:5′-CCCTGTCACCAACATTTGC-3′ R:5′-AGAAGTGTGGGATCCGCTTG-3′	90	60
β	F:5′-CTCTGGATGATCAGGTCATATTGCT-3′ R:5′-GCCATCTCG AACATCAATGGA-3′	92	60
γ	F:5′-GGGAAGCTGTGCAAGAAGAAA-3′ R:5′-TGGTAGCACATTCTGCCTCACT-3′	69	60
CYP26A1	F:5′-GAGACCCTTCGACTGAATCC-3′ R:5′-GGAGGTCCATTTAGAAGCTGC-3′	332	56
CRABP-I	F: 5′-GCCATGCTGAGGAAAGTG-3′ R: 5′-TTCTCCGACCTTGAAGTTG-3′	132	65
II	F: 5′-ATGCCCAACTTCTCTGGCAA-3′ R: 5′-CGTCATGGTCAGGATCAGTT-3′	375	59
NGN1	F: 5′-TCAGCAGGCAATAGATTGGG -3′ R: 5′-AAAGGAAAGGCCGTCTAGGG-3′	200	58
β-III tubulin	F: 5′-CTCAGGGGCCTTTGGACATC-3′ R: 5′-CAGGCAGTCGCAGTTTTCAC-3′	160	60
Primers for bisulfite sequencing PCR			
CRABP-I	F:5′- AGGGAGGTGGAGGTTTTTTAGT -3′ R:5′- ACCAACTTACCCAATACCTTAAAC-3′	359	53
CRABP-II	F:5′-GGGTTTTTGTTTAATTTTTTAATGTT-3′ R:3′-AACTAAATCCAATAAAACCACTCC-5′	214	58