Gene 554 (2015) 174–180

Contents lists available at ScienceDirect

Gene

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

Human L-DOPA decarboxylase mRNA is a target of miR-145: A prediction to validation workflow

Emmanuel I. Papadopoulos, Emmanuel G. Fragoulis, Andreas Scorilas *

Department of Biochemistry and Molecular Biology, University of Athens, Athens 15701, Greece

ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form 16 October 2014
Accepted 25 October 2014
Available online 29 October 2014

Keywords:
L-DOPA decarboxylase
Experimental validation
MicroRNAs
Real-time PCR
Target prediction algorithms

ABSTRACT

L-DOPA decarboxylase (*DDC*) is a multiply-regulated gene which encodes the enzyme that catalyzes the biosynthesis of dopamine in humans. MicroRNAs comprise a novel class of endogenously transcribed small RNAs that can post-transcriptionally regulate the expression of various genes. Given that the mechanism of microRNA target recognition remains elusive, several genes, including *DDC*, have not yet been identified as microRNA targets. Nevertheless, a number of specifically designed bioinformatic algorithms provide candidate miRNAs for almost every gene, but still their results exhibit moderate accuracy and should be experimentally validated. Motivated by the above, we herein sought to discover a microRNA that regulates *DDC* expression. By using the current algorithms according to bibliographic recommendations we found that miR-145 could be predicted with high specificity as a candidate regulatory microRNA for *DDC* expression. Thus, a validation experiment followed by firstly transfecting an appropriate cell culture system with a synthetic miR-145 sequence and sequentially assessing the mRNA and protein levels of *DDC* via real-time PCR and Western blotting, respectively. Our analysis revealed that miR-145 had no significant impact on protein levels of *DDC* but managed to dramatically downregulate its mRNA expression. Overall, the experimental and bioinformatic analysis conducted herein indicate that miR-145 has the ability to regulate *DDC* mRNA expression and potentially this occurs by recognizing its mRNA as a target.

1. Introduction

L-DOPA decarboxylase (DDC) was the first decarboxylase which presence was experimentally confirmed in mammals in the late 1930s (Holtz et al., 1938). Since then, DDC has been detected in several tissues of these organisms indicating a potential implication of this enzyme in various biological processes (Zhu and Juorio, 1995). In humans, DDC has been isolated and biochemically characterized as the enzyme that is responsible for the conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine, and putatively for this of 5-hydroxytryptophan (5-HTP) to serotonin (Mappouras et al., 1990). Human DDC can be subjected to multiple regulatory mechanisms at various levels. Similar to data emerged from studies in insects (Poulikakos et al., 2002) and rodents (Poulikakos et al., 2001) human DDC can be either targeted in the membranes or found in the cytosol of the cells (Chalatsa et al., 2011). In parallel, its enzymatic activity has proven to be affected by various factors, including specific inhibitors (Vassiliou et al., 2009). Likewise, the expression of *DDC* can be widely regulated in human cells. Two different alternative transcripts that share the same coding region but differ in the largest part of their 5′-untranslated region (UTR) have been reported to encode the full-length isoform of DDC that consists of 480 amino acids (Ichinose et al., 1992). Alternative usage of two distinct promoters located to the 5′ flanking region of two alternative exons 1 has been found to be responsible for the generation of the aforementioned transcripts (Ichinose et al., 1992). Alternative splicing may also occur within the coding region of *DDC*, as one transcript that lacks exon 3 sequence (O'Malley et al., 1995) and another one that lacks the corresponding sequences of exons 10–15 including though that of an alternative exon 10 (Vassilacopoulou et al., 2004) have been likewise detected. Furthermore, the mRNA levels of *DDC* seem to be also subjected to strict regulation, as relative aberrations have been correlated with various types of cancer (Kontos et al., 2010; Koutalellis et al., 2012).

A landmark event that recently occurred in the field of molecular biology was the discovery of microRNAs (miRNAs). miRNAs comprise a class of small non-coding RNAs that are endogenously transcribed and subsequently loaded onto the RNA-induced silencing complex (RISC). The latter is a ribonucleoprotein complex, where miRNAs function as the guide molecules that lead RISC to specific mRNAs, which constitute their targets (Ha and Kim, 2014). The number of the potential mRNA-targets for a given miRNA may exceed 100 and vice

Abbreviations: DDC, L-DOPA decarboxylase; L-DOPA, 3,4-dihydroxyphenylalanine; 5-HTP, 5-hydroxytryptophan; miRNA, microRNA; RISC, RNA-induced silencing complex; 3′-UTR, 3′-untranslated region; FBS, fetal bovine serum; PAP, poly(A) Polymerase; SNORD48, small nucleolar RNA, C/D box 48; CCNA2, cycline A2.

* Corresponding author at: Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Panepistimiopolis, Athens 15701, Greece.
E-mail address: ascorilas@biol.uoa.gr (A. Scorilas).

http://dx.doi.org/10.1016/j.gene.2014.10.043
0378-1119/

versa (Krek et al., 2005). miRNAs seek their targets by mainly scanning the 3′-UTR of the mRNAs for complementary sequences. Once they find their targets they induce either repression of translation or deadenylation and sequentially decay of the mRNA, whereas recent studies claim that the above mechanisms might also occur simultaneously (Meijer et al., 2013; Guo et al., 2010; Hu and Coller, 2012). The precise rules that govern this interaction have not been elucidated yet, as miRNAs show partial or even limited complementarity with their targets. Remarkably, the majority of the verified miRNA–mRNA interactions exhibit perfect base-pairing only in the region that corresponds to the nucleotides 2 to 8 of the 5′-end of the miRNA, which is known as the seed region. Few mismatches in this site have been also reported though, but they are usually compensated by the 3′-end of the miRNA sequence which partially hybridizes with the target-sequence in this case (Pasquinelli, 2012).

Overall, the above situation raised a lot of practical difficulties regarding the identification of miRNA targets. Nonetheless, researchers tried to resolve this issue by developing sophisticated computational tools that predict candidate targets for miRNAs and adequate experimental methodologies that can test the validity of these predictions (Thomson et al., 2011; Kuhn et al., 2008). From a computational perspective, the bioinformatic tools used in miRNA target prediction are divided into the ab initio and the machine learning algorithms (Reyes-Herrera and Ficarra, 2012). Although their design is based on the same principles, these tools differ in the workflow that has been followed for the generation of a scoring system which determines whether a miRNA–mRNA reaction could truly occur or not (Yousef et al., 2009; Yue et al., 2009). In computational science, this represents a binary classification problem usually addressed by applying the process of supervised learning (Yousef et al., 2009). The latter involves the implementation of a process called training of the algorithm which can be achieved with the assistance of a data set demonstrating an under-investigation feature. The aim of supervised learning is to determine via statistical analysis the distribution of specific parameters considered to be responsible for the generation of the desired feature within the training data set and accordingly produce an inferred function that could be used for predicting the behavior of unknown data regarding the same feature (Yue et al., 2009). In ab initio algorithms, the training data consist of a set of miRNAs along with their experimentally validated targets. These interactions are carefully examined in silico by the designer of the algorithm who is responsible for the production of the inferred function. On the contrary, machine learning algorithms require two training sets; a positive that consists of sets of miRNAs and verified targets, and a negative which includes unfavored miRNA–mRNA interactions, usually assessed by experimental data. The role of the algorithm designer in this case is limited to the selection of the parameters that will be assessed by the program, which automatically estimates the validity of each one and proceeds to the development of the evaluation formula. Ab initio algorithms appeared before their machine learning counterparts. The main parameters evaluated in ab initio algorithms are the complementarity in seed region, the thermodynamic stability of the reaction and the conservation of target-sequence. Machine learning algorithms also examine the first two parameters, as well as multiple structural features of the interacting sequences, but do not usually evaluate conservation (Reyes-Herrera and Ficarra, 2012; Yousef et al., 2009; Yue et al., 2009).

So far none of the known miRNAs has been found to recognize *DDC* as a target. Thus, the goal of this study was to take advantage of the already existing tools and develop a workflow which could lead us to the discovery of a miRNA that exerts regulatory action on *DDC* expression.

2. Materials and methods

2.1. Bioinformatic tools

The open access web-based databases of the experimentally validated miRNA targets, miRecords (http://mirecords.biolead.org/) (Xiao et al., 2009) and TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) (Vergoulis et al., 2012) plus 17 miRNA target prediction algorithms (Table 1) were accessed during this study. All algorithms were used either by applying their default parameters or by accessing the pre-created lists of predictions provided in their websites.

2.2. Cell culture

The prostate cancer cell line LNCaP was cultured in a humidified incubator at 37 °C and under 5% CO_2, using RPMI 1640 (PAA Laboratories, Pasching, Austria), supplemented with 10% fetal bovine serum (FBS), 100 kU/L penicillin, and 0.1 g/L streptomycin as a culture medium.

2.3. RNA transfection

A synthetic sequence of human miR-145 (Dharmacon, Lafayette, CO) was transfected into LNCaP cells by using the X-tremeGENE siRNA Transfection Reagent (Roche, Mannheim, Germany) according to the manufacturer's instructions. The process was executed in 6-well plates, where LNCaP cells were seeded in a density of 10^5 cells/cm^2. Prior to transfection, LNCaP cells were left inside the incubator for 24 h in order to adhere to the culture vessel and adapt to the culture environment. The final concentration of the synthetic miRNA added to the culture medium was adjusted to 100 nM. According to manufacturer's

Table 1
Human microRNA target prediction algorithms.

Algorithm	Website	Reference
DIANA-microT-ANN	http://diana.cslab.ece.ntua.gr/DianaTools/index.php?r=microtv4/index	Reczko et al. (2012)
EIMMo	http://www.mirz.unibas.ch/ElMMo2/	Gaidatzis et al. (2007)
GenMiR++	http://www.psi.toronto.edu/genmir/	Huang et al. (2007)
MicroInspector	http://mirna.imbb.forth.gr/microinspector/	Rusinov et al. (2005)
MiRanda-mirSVR	http://www.microrna.org/microrna/getGeneForm.do	Betel et al. (2010)
miREE	http://didattica-online.polito.it/eda/miREE/	Reyes-Herrera et al. (2011)
MiTarget2-miRDB	http://mirdb.org/miRDB/	Wang and El Naqa (2008)
PicTar	http://pictar.mdc-berlin.de/	Krek et al. (2005)
PITA	http://genie.weizmann.ac.il/pubs/mir07/index.html	Kertesz et al. (2007)
RepTar	http://bioinformatics.ekmd.huji.ac.il/reptar/	Elefant et al. (2011)
RNA22	https://cm.jefferson.edu/rna22v2.0/	Miranda et al. (2006)
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/	Krüger and Rehmsmeier (2006)
STarMir	http://sfold.wadsworth.org/cgi-bin/starmir.pl	Long et al. (2008)
SVmicro	http://compgenomics.utsa.edu/svmicro.html	Liu et al. (2010)
TargetMiner	http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm	Bandyopadhyay and Mitra (2009)
TargetScanHuman	http://www.targetscan.org/	Garcia et al. (2011)
TargetSpy	http://www.targetspy.org/	Sturm et al. (2010)