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Construction of constitutive expression systems of microRNAs from the genomic region DLK1-DIO3 with putative tumor suppressor role

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Abstract

Thyroid carcinoma is the most common endocrine cancer in the world. Over the last years attention has been focused on the role of microRNAs (miRs), small non-coding RNAs molecules, in the tumoral thyroid cell biology, especially in the diagnosis and prognosis of the papillary histotype (PTC). In a recent study of the group, the expression of miRs from the DLK1-DIO3 genomic region has been shown to be decreased in PTC. Importantly, distinct members from this genomic region have already been described as tumor suppressor miRs in several types of cancer. The aim of the project herein was to identify, based on target prediction and gene enrichment analysis, candidate miRs with tumor suppressor potential and to generate DNA plasmid constructions. MiR-495-3p and miR-485-5p were identified as the candidates with the highest tumor suppressor potential role in PTC among more than 50 miRs from the DLK1-DIO3 region. The construction of expression systems containing the miRs was performed based in mollecular clonning methodology and will certainly contribute to further functional validation of the role of these miRs in PTC.

Key words:

Molecular clonning, Thyroid cancer, microRNAs

Introduction

The papillary thyroid carcinoma (PTC) is the most prevalent histotype of thyroid cancer, comprising more than 80% of the cases in the United States[1]. Recently, the influence of miRs, small RNA molecules that play an important role in post-transcriptional regulation, has been demonstrated in tumorigenesis in virtually all types of cancer. MiR regulatory role rely on its potential to target mRNAs for cleavage or translational repression, based on nucleotide complementarity[2].

In PTC, several miRs have been reported to present oncogenic or tumor suppressor roles, including members of the cluster of DLK1-DIO3 region[3]. Our group recently identified a global decrease in the expression of the miRs of the DLK1-DIO3 region in PTC but the influence of this event in this type of cancer lacks further elucidation[4]. The role of DLK1-DIO3 miR cluster in PTC is reinforced by studies regarding the Temple Syndrome that show that a total or partial deletion of this region results in higher chances of premature thyroid cancer development[5].

Systems of constitutive expression have been successfully used to demonstrate the influence of the modulation of a single miR in tumorigenesis and cancer progression. Therefore, we aimed to construct systems for constitutive expression of selected miRs of the referred cluster to posteriorly investigate the effects of its modulation in tumorigenesis and PTC progression.

Results and Discussion

The establishment of PTC cell lines that overexpress miRs in a constitutive way is essential to make it possible to observe the effects on the modulation of these molecules in long term and *in vivo*.

Using bioinformatic tools, we ranked the miRs of the DLK1-DIO3 genomic region according with its potential of interacting with cancer related targets. The analysis pointed hsa-miR-485 and hsa-miR-495 as the candidates with the highest score based on predicted interactions with potential targets (Image 1).

Using Genome Browser, the genomic regions flanking the selected candidate miRs were identified and Forward (Fw) and Reverse (Rev) oligonucleotides were designed for each gene. To assure correct orientation of the insert within plasmid, sites for *HindIII* and *EcoRI* restriction enzymes were included in Fw and Rev primers, respectively. The resulting PCR products were then double-digested and ligated with *pZsYellow1-C1* and *pAmCyan1-C1* plasmids. The generation of stable miR-485 and -495 -expressing models will be of great importance for further *in vitro* and *in vivo* functional investigation of these genes in PTC.

A

	cancer	global
hsa-miR-495-3p	62	1371
hsa-miR-485-5p	61	1487
hsa-miR-665	56	1245
hsa-miR-654-5p	50	946
hsa-miR-539-5p	50	1156
hsa-miR-543	47	976
hsa-miR-381-3p	41	828
hsa-miR-541-3p	41	855
hsa-miR-300	40	825
hsa-miR-544a	38	858
hsa-miR-656-3p	36	715
hsa-miR-494-3p	33	644
hsa-miR-329-3p	32	654
hsa-miR-655-3p	32	669
hsa-miR-134-5p	30	637
hsa-miR-369-3p	30	531
hsa-miR-493-5p	29	643
hsa-miR-382-5p	27	581
hsa-miR-377-3p	26	680

B

	oncogenes	global
hsa-miR-495-3p	41	1371
hsa-miR-485-5p	37	1487
hsa-miR-300	35	825
hsa-miR-381-3p	35	828
hsa-miR-665	34	1245
hsa-miR-494-3p	33	644
hsa-miR-410-3p	31	665
hsa-miR-1185-1-3p	30	208
hsa-miR-1185-2-3p	30	208
hsa-miR-543	29	976
hsa-miR-329-3p	27	654
hsa-miR-493-5p	26	643
hsa-miR-654-5p	26	946
hsa-miR-539-5p	26	1156
hsa-miR-889-3p	26	298
hsa-miR-541-3p	26	855
hsa-miR-544a	25	858
hsa-miR-656-3p	25	715
hsa-miR-668-3p	24	405

Image 1. Ranking of DLK1-DIO3 miRs according to bioinformatic prediction of targets. A. A. Figure shows the 20 miRNAs of the DLK1-DIO3 region with the highest numbers of predicted cancer-related target genes (left column) and the numbers of its global predicted targets (right column). B. Figure shows the 20 miRNAs of the DLK1-DIO3 region with the highest numbers of predicted target genes considered oncogenes (left column) and the numbers of its predicted global targets (right column).

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