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Down-regulation of dicer expression in ovarian cancer tissues

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Abstract

Objectives: Although numerous recent studies have focused on analyses of microRNA expression profiles in cancer cells, the expression patterns of the enzymes responsible for the generation of miRNAs remains largely unexplored. The purpose of this study was to investigate whether *Dicer* mRNA expression is altered during progression of ovarian cancer.

Design and methods: Total RNA was extracted from ovarian tissue specimens (normal, benign and malignant tumors). The expression of *Dicer* was analyzed by semi-quantitative RT-PCR.

Results: We analyzed a total of 34 ovarian tissue samples and found that *Dicer* mRNA expression is down-regulated in the majority of ovarian tumors when compared to normal tissues.

Conclusions: Our results suggest that the levels of *Dicer* mRNA should be evaluated as a potential new candidate biomarker for ovarian cancer.

Keywords: Dicer; ovarian cancer; biomarker

Introduction

In recent years, the regulation of gene expression by post-transcriptional mechanisms mediated by miRNAs has been well-established. Dicer is a cytoplasmic RNase III type endonuclease, an essential protein component of the microRNA machinery with a key function in the generation of miRNAs and siRNAs. Further, it is implicated in the assembly of the RNA-induced silencing complex (RISC) [Ref. [1] provides a detailed review]. MicroRNAs are tiny RNA molecules with length of approximately 22 bp that regulate pathways that are essential to most biological processes, including differentiation, development, and apoptosis. Given that many of these pathways are aberrantly altered in cancer cells, numerous recent studies have revealed the great importance of these tiny non-coding RNAs for cancer development and progression. Therefore, an intensive effort has been devoted to understand the versatile roles of miRNAs in cancer as well as to identify their aberrant expression profiles in tumor cells (miRNone) and their correlation with varying degrees of malignancy [1]. In this respect, several miRNAs have identified as being deregulated in ovarian cancer [1,2]. However, although Dicer is essential for the production of miRNAs, its expression in cancer cells has not been studied in depth. In this study, the expression of *Dicer* was analyzed by semi-quantitative RT-PCR in clinical specimens from normal, benign and ovarian cancer tissues. *Dicer* was found down-regulated in the vast majority of ovarian cancer and benign samples as compared to normal tissues.

Materials and methods

Patients and clinical specimens

Tissue specimens used in this study were collected at the University of Turin, Italy. Tissues were obtained after patients'

Abbreviations: miRNAs, microRNAs; siRNAs, small-interfering RNAs.

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written approval consent under a general tissue collection protocol approved by the Institutional Review Board and the University of Turin. Samples were snap-frozen at liquid nitrogen and stored at −80 °C.

RNA extraction and RT-PCR

Tissue samples were pulverized under liquid nitrogen with a pestle and mortar. Approximately 20–30 mg of tissue was used for RNA extraction. RNA was extracted with RNeasy (Qiagen, Valencia, CA) and treated with DNase I according to manufacturer's instructions (Qiagen). The integrity and quality of RNA was confirmed by agarose gel electrophoresis and absorbance readings at 260 nm. RT-PCR was carried out using OneStep (Qiagen), 500 ng total RNA template (100 ng for β-*actin*) and the following conditions: 50 °C for 30 min to reverse transcribe the RNA, 95 °C for 15 min to inactivate reverse transcriptases, denature the cDNA template and activate HotStar Taq, followed by 94 °C for 1 min, 55 °C (60 °C for β-*actin*) for 1 min, 72 °C for 1 min, for 28 cycles (30 cycles for β-*actin*), and a final extension step of 72 °C for 10 min. For amplification of *Dicer* the following primers were used: 5′-CAAGTGTCAGCTGTCAGAACTC-3′ (forward) and 5′-CAATCCACCACAATCTCACATG-3′ (reverse) [3] and for β-*actin*: 5′-ACAATGAGCTGCGTGTGGCT-3′ (forward) and 5′-TCTCCTTAATGTCACGCACGA-3′ (reverse). Equal volumes of PCR products were resolved on agarose gel, visualized with ethidium bromide, and photographed. Images were analyzed with GelDoc (Kodak). The intensity of *Dicer* signal was normalized for the intensity of β-*actin*.

Data analysis

For normalization of PCR product intensity the β-*actin* housekeeping gene was used. A threshold was manually considered in a way that maximum discrimination of differential *Dicer* expression was achieved. The statistical significance was determined with the Fischer exact test.

Results and discussion

Although numerous studies have been published on the potential roles and the aberrant expression profiles of miRNAs in cancer cells [reviewed in 1], it remains largely unknown whether the expression levels of Dicer are altered in tumor cells. In the present capsule we examined the levels of *Dicer* mRNA expression in ovarian cancer using RT-PCR. We used 10 normal ovary tissue specimens, 8 benign, and 16 ovarian cancer specimens. The characteristics of the ovarian tissue specimens are shown in Table 1. As shown in Figs. 1A and B, significant down-regulation of *Dicer* expression was observed in ovarian cancer when compared to normal and benign tissues. The intensity signals in pixels for *Dicer* were divided with the respective signal for β-*actin* (*Y*-axis) and plotted versus the sample number (*X*-axis), as shown in Fig. 1B. We set up an arbitrary threshold of 0.6 (ratio of *Dicer* vs. β-*actin*). Based on presented data, 3/10 of normal, 5/8 benign, and 12/16 cancer samples, are below this limit, indicating that *Dicer* mRNA is reduced in both benign and cancerous tumor tissues relative to normal tissues. Comparison of normal with benign and cancer samples indicates that these results are significant (Fisher exact test $p=0.034$). Further, based on the data in Table 1 we observed marked down-regulation of *Dicer* expression in tumors of higher grade (Grade III, 6/7) and higher stage (Stage III, 6/8).

Previous studies indicated that decreased expression of *Dicer* is associated with decreased survival in patients with non-small cell lung cancer [4,5]. Contrary, higher *Dicer* mRNA expression has been associated with poor prognostic factors in esophageal cancer [6]. In metastatic prostate adenocarcinoma, increased Dicer levels were detected both by immunohistochemistry and gene array analysis [7]. Recently, two different studies were performed in ovarian cancer, however, with contradictory results [8,9]. In the first study, Dicer was found up-regulated in cases of ovarian serous carcinoma [8], while according to the second study, *Dicer* mRNA and levels of the corresponding protein were found down-regulated in 60% of ovarian cancer cases, and lower Dicer expression was associated with advanced tumor stage [9]. Further, a recent analysis of *Dicer* mRNA and protein expression in early- and late-stage epithelial ovarian cancer reported no significant changes, however, no correlation with normal samples was performed [2]. Notably, a gain in copy number for the gene encoding Dicer in ovarian cancer (24.8% of samples examined) was reported by the same group although the effects on Dicer expression levels were not examined [10]. Our results are in accordance with a recent report on decreased *Dicer* expression in ovarian cancer specimens [9]. More significantly, we found lower *Dicer* expression in more advanced stages of ovarian cancer. This original observation merits to be further evaluated and validated in a larger cohort of patients.

Table 1
Association of Dicer expression in ovarian cancer patients, benign and normal controls with clinicopathological features.

		Cases	High expression	Low expression
Normal				
Age	≤60	7	5	2
	>60	3	2	1
Benign				
Age	≤60	5	3	2
	>60	2	2	0
	U	1	1	0
Tumor				
Age	≤60	5	2	3
	>60	11	2	9
Grade	0	1	0	1
	1	2	0	2
	2	5	2	3
	3	7	1	6
	U	1	1	0
Stage	I	5	1	4
	II	1	0	1
	III	8	2	6
	U	2	1	1

U: unknown or not reported.

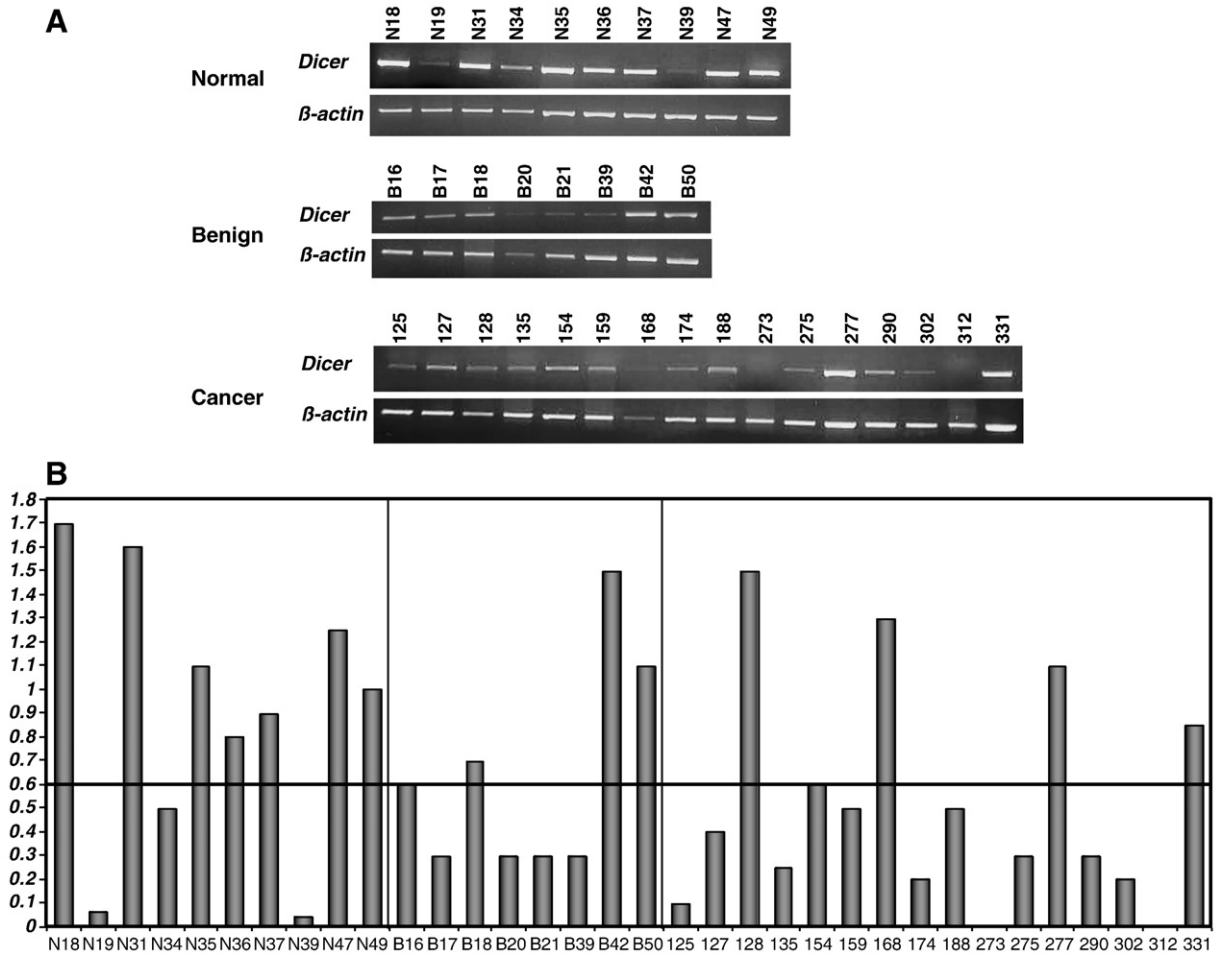

Fig. 1. (A) RT-PCR analysis of *Dicer* and β-*actin* mRNA expression in ovarian tissue specimens. (B) Normalization of *Dicer*-specific PCR product intensity over β-*actin* (*Y*-axis). The threshold was arbitrary set to 0.6. On the *X*-axis, N denotes normal, B benign and numbers represent ovarian cancer patients. Vertical lines separate normal from benign and benign from cancer samples.

The basis of the observed down-regulation of *Dicer* expression in ovarian tumors is unknown. Biocomputational analysis of the *Dicer1* gene (GenBank accession number NM_177438) using the CpGplot/CpGreport (EBI, European Bioinformatics Institute), at http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html revealed a very strong prediction for a CpG island that spans the first exon of the gene at position −587 to +682, with a C+G content of 73.05%, 147 CpG dinucleotides and an observed/expected ratio of 0.93, with a threshold of >0.6 the limit being considered significantly high (data not shown). The presence of this strong CpG island indicates that genomic DNA methylation may account for *Dicer* down-regulation in ovarian tumors, a possibility that merits further investigation. Chiosea et al. [5] have reported loss of heterozygosity of the *Dicer* gene locus on 14q32.31-32, in 1/6 cases of lung adenocarcinoma. In breast cancer, utilization of alternative gene promoters was demonstrated to regulate transcription of *Dicer* [11]. All the above indicate that the mechanism(s) underlying the aberrant regulation of *Dicer* expression in tumor cells are complex and probably involve a combination of genomic alterations, epigenetic modifications, and alternative promoter usage. Delineation of the molecular mechanisms underlying the observed down-regulation of *Dicer* in tumor cells presents significant interest, given the emerging roles of the microRNA machinery in cancer.

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