miR-183 as a molecular and protective biomarker for cancer in schizophrenic subjects

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Abstract. Previous studies have suggested that schizophrenia is associated with a reduced risk of cancer. Genes that are involved in cell cycle regulation seem to have additional functions in post-mitotic neurons involved in neuronal migration and synaptic plasticity. MicroRNAs (miRNAs) play a dominant role in the regulation of gene expression in the central nervous system (CNS). Due to their involvement in a large number of CNS pathways, miRNAs pose as appealing molecules for further investigation, with potential diagnostic, prognostic and therapeutic value. In the present study, we investigated the potential association between cancer and schizophrenia in 2 patient sample groups. We analyzed a large number of miRNAs in a control group of 6 schizophrenic patients and a study group of 8 schizophrenic patients with a solid tumor. A comparison between the control and study groups showed that only miR-183 was differentially expressed. Specifically, a significant downregulation of miR-183 in the samples of the study group was observed. Although a larger sample size is required to validate this result for the general patient population, our findings provide a first indication that miR-183 may play a role in regulating the expression of other genes with onco-suppressor activity. Our results are in agreement with the theory that patients with schizophrenia may have a tumor suppressor gene or enhanced neuronal apoptotic activities. Further studies are required in order to shed light on the role of miRNAs and particularly, on the suppressive role of miR-183 in the neurobiological pathways involved in schizophrenia.

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Introduction

Schizophrenia and cancer are disorders characterized by a broad spectrum of clinical phenotypes and complex genetics. The hypothesis that the incidence of cancer in patients with schizophrenia is reduced has been confirmed in clinical reports, although this hypothesis has been reported and formulated since 1909 (Commissioners in Lunacy for England and Wales: Annual Report. London. HMSO, 1909). Since then, even though there are conflicting results on this hypothesis, in some population-based incidence studies a decreased cancer risk has been found in patients with schizophrenia (1-5). Furthermore, in a recent study, cancer risk was associated with the duration and age of onset of schizophrenia in a large sample of patients with schizophrenia and bipolar disease and specifically, duration was inversely correlated with cancer risk (6).

Schizophrenia and cancer are heterogeneous syndromes of different disorders that share clinical symptoms and features (7). Both conditions are mediated by common etiological factors; genetic and environmental factors play a distinctive role in the development of both syndromes. The development of cancer is characterized by increased gene expression that leads to uncontrolled cell proliferation, whereas the development of schizophrenia is characterized by the reduced expression of genes whose products suppress cellular proliferation (tumor suppressor gene activity) (8,9) and increase the rate of apoptosis (10).

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that play an important role in various biological processes (11). Bioinformatics have predicted that approximately one-third of human genes are targeted by miRNAs. There is increasing evidence demonstrating the involvement of miRNAs in human cancer. Previous studies have revealed that 98 out of 186 miRNA genes located in cancer-associated

genomic regions may frequently be found in different types of tumor, whereas the altered expression of let-7 and miR-155 in lung cancer has been shown to correlate with patient survival (12,13). Additionally, a number of studies have shown that the pathogenesis of schizophrenia may be related to the dysregulation of miRNA expression (14,15).

In this study, we investigated the potential role of miRNAs in the development of cancer in 2 groups of patients with schizophrenia, in an attempt to provide further evidence for the low incidence of cancer risk among schizophrenic patients. The 1st group consisted of patients with schizophrenia, whereas the 2nd group included patients with schizophrenia and a concomitant diagnosis of a solid tumor.

Materials and methods

Subjects. Six patients (male/female, 2/4) (study group) were recruited from the Psychiatric Hospital of Attika and from the Oncology Outpatient Department of the University General Hospital 'Attikon' between February and June 2011. Patients were assessed according to the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV) criteria (16) and by Positive and Negative Syndrome subscales (PANSS) (17). Exclusion criteria included a history of any neurological disease and current substance misuse or dependence in the preceding 6 months as defined by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [American Psychiatric Association (APA), 1994]. All patients were in a stabilized psychological state. The diagnosis of cancer was made according to the medical records of the patient. It should be noted that it is extremely difficult to find patients with this type of co-morbidity (schizophrenia and cancer).

The second group of patients (control group) consisted of 8 schizophrenic patients (male/female, 1/7), which had no evidence of cancer disease. Inclusion criteria for the control group were an age of 30-70 years, body weights within 10% of an appropriate body mass index, no other serious diseases other than schizophrenia, no clinically significant abnormal laboratory values and no pathological findings upon thorough clinical examination. Additionally, individuals in the control group had no history of hypersensitivity (asthma, urticaria and eczema), autoimmune disorders, such as systemic lupus erythematosus, uncontrolled hypertension or serious heart, lung, liver or renal conditions.

All patients were in a stabilized psychological state and their medication had not been altered over a period of at least 6 months pior to enrollment in this study. Patients with a smoking history were included in this study. However, exclusion criteria included a recent history (>1 year) of alcoholism, use of recreational drugs or drug addiction. No consumption of alcohol was allowed within 48 h prior to blood collection. All female participants were tested for pregnancy and the results were negative. All patients were on regular medical treatment with antipsychotics. All participants were able to communicate effectively, were informed of the nature of the study and provided written informed consent. The study was approved by the institutional review board and ethics committee of both participating hospitals and was conducted in accordance with Good Clinical Practice principals and applicable local regulations. The patients in the control group were clinically followed-up for 1 year after blood collection and showed no evidence of cancerous disease.

Sample collection and preservation for microarray analysis. miRNA profiling for each patient was performed on whole blood samples. Preservation of the gene expression status of the samples was achieved by collecting 500 μl of whole blood from each patient to an RNAprotect Animal Blood Tube (Qiagen, Hilden, Germany). Following gentle inversion of the tubes for 8-10 times, the tubes were incubated for 2 h at ambient temperature, according to the manufacturer's instructions, to allow for efficient cell lysis. All tubes were then stored at -70˚C prior to RNA purification.

RNA purfication and miRNA microarray analysis. Total RNA purification that contained small RNA, including miRNA, was carried out using the miRNeasy Protect Animal Blood kit (Qiagen). All further experiments that included sample RNA quality control and miRNA profiling were conducted by Exiqon Services (Vedbaek, Denmark). Briefly, RNA quality control and measurement were carried out using an Agilent 2100 Bioanalyzer and a nanodrop instrument. All samples and a reference RNA sample, labeled with Hy3™ and Hy5™ fluorescent labels, respectively, were mixed pair-wise and hybridized to the miRCURY LNA™ miRNA Array 6th gen (Exiqon), which contains capture probes targeting all miRNAs for humans, mice or rats registered in the miRBASE 16.0. The hybridization was performed using a Tecan HS4800™ hybridization station (Tecan, Grödig, Austria). The miRCURY LNA™ miRNA Array slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc., Santa Clara, CA, USA) and the image analysis was carried out using the ImaGene® 9 (miRCURY LNA miRNA Array Analysis Software, Exiqon).

Statistical analysis. Principal component analysis was used in order to explore, based on the expression profile, the naturally arising sample classes. It was revealed that the samples cluster according to their biology. A statistical analysis of the miRNA expression between the control and study group was also carried out by Exiqon; The Student's t-test was used for determination of the statistical significance of the relative expression of miRNAs between the 2 groups and the Bonferroni multiple testing adjustment method was subsequently applied to the P-values for the control of possible false positive results.

Results

In total, 345 different miRNAs were analyzed by the miRCURY LNA miRNA Array. Fig. 1 shows a heat map diagram that depicts the expression of the 50 miRNAs with the highest standard deviation on all samples. Statistical analysis of the results showed that only miR-183 showed a significantly differential expression between the 2 groups of patients ($P<0.05$) and specifically, a significantly greater level of miR-183 expression was recorded for the control group of patients. The specific result suggested the possibility that the expression level of miR-183 may be directly related to the absence of a solid tumor in the presence of schizophrenia.

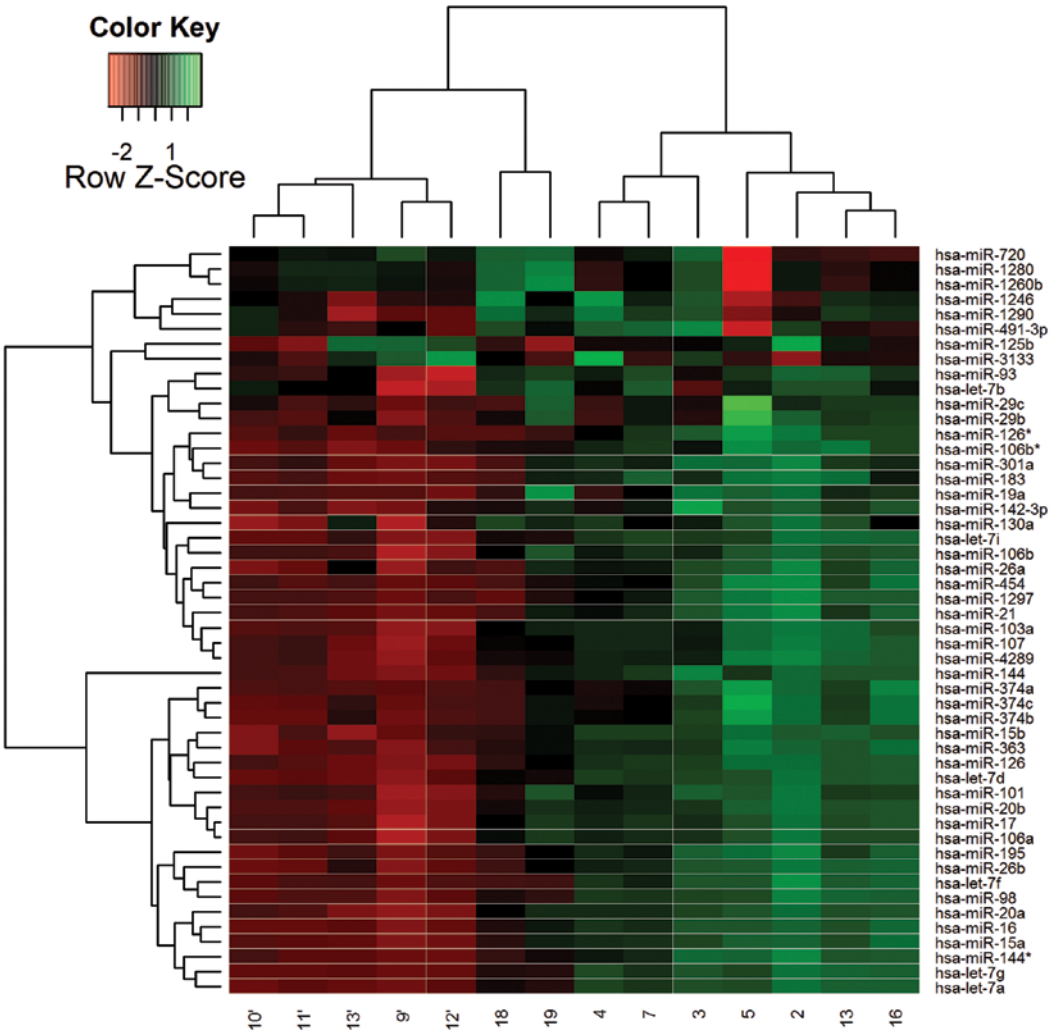

Figure 1. Heat map diagram showing the expression of the 50 miRNAs with the highest standard deviation in all samples. The color scale illustrates the relative expression level of miRNAs and specifically, red color represents an expression level below the reference channel, whereas green color represents an expression higher than the reference. Samples 9', 10', 11', 12', 13', 16, 18 and 19 correspond to the study group of patients (cases of schizophrenia with tumor formation), whereas samples 2, 3, 4, 5, 7 and 13 correspond to the control group of patients (cases of schizophrenia without tumor formation). The expression of hsa-miR-183, which was found to be significantly higher in the samples of the control group of patients, is also indicated.

Discussion

The hypothesis that schizophrenia is associated with a reduced risk of cancer has been addressed in a number of studies; however, there are conflicting results. The implication of several biological characteristics such as the wingless-related family of proteins (Wnt) pathways of inactivation, dopamine effects and enhanced natural killer cell activities has been reported in clinical studies (10,18). Furthermore, antipsychotic agents have been proposed to account for the reduced cancer risk in patients with schizophrenia in several studies (1,19). Specifically, a high dose of phenothiazine has been shown to reduce the risk of prostate cancer, whereas another study reported that the long-term use of antipsychotics may decrease the risk of rectal, colon and prostate cancer (20). Thus, the anticarcinogenic effect of antipsychotic agents should be considered for the decreased cancer risk among patients with chronic schizophrenia.

miRNAs, as mentioned above, are emerging as important mechanisms implicated in the modulation of gene expression (21), and are thus genetic factors contributing to the etiology of both psychiatric disorders and cancer. It is estimated that miRNAs regulate <30% of human gene expression at the post-transciptional and translational levels (11). Moreover, miRNAs are involved in the modulation of a wide range of biological processes, including programmed cell death (apoptosis and autophagy) (22-24) and those that are expressed in the brain affect neuronal differentiation, synaptosomal complex localization and synapse plasticity, all functions thought to be disrupted in schizophrenia (25). In the present study, we investigated miRNA expression in plasma from a sample of patients with schizophrenia and another sample of patients with schizophrenia and cancer. In this way, we investigated the possible role (positive or negative) of miRNAs in the development of cancer in patients with schizophrenia, in an attempt to obtain evidence regarding their role cancer protection (onco-suppressor activity). Our results showed an overexpression of miR-183 in the group of schizophrenic patients without a history of cancer. On the contrary, the absence of miR-183 expression in the group of patients with schizophrenia and cancer may be an indication that this miRNA is a protective factor against cancer.

miR-183 has recently been implicated in the modulation of different stages of apoptotis and autophagy through the regulation of apoptosis and autophagy-related genes (24,26,27). Specifically, the knockdown of miR-183 expression has been shown to induce autophagic cell death in medullary thyroid cancer, through the regulation of certain tumor suppressive signaling pathways, indicating that miR-183 may be an attractive therapeutic target (28). A recent study reported that the overexpression of miR-183 correlated with the metastatic potential of lung cancer cells (29). Furthermore, the overexpression of miR-183 has been shown to inhibit the migration and invasion of lung cancer cells. The oncogenic role of miR-183 has been revealed in a recent study by targeting the transcription factor, EGR1, and promoting tumor cell migration in different types of cancer, such as sarcomas and colon tumors (30). Thus, miR-183 may play a tumor suppressor role, possibly by activating the expression of tumor suppressor genes that control cell differentiation or apoptosis (31). The onco-supressor role of miR-183 is indicated in the study by Zhu *et al* (32), who demonstrated that the downregulation of miR-183 promotes the migration and invasion of osteosarcoma cells by targeting Ezrin. Moreover, it has recently been shown that Tiam1, that is downregulated by miR-183, presents an overexpression pattern in ovarian cancer cells. Particularly, there seems to be an implication of Tiam1 in the migration, invasion, viability and, in general, in the aggressive profile of ovarian cancer cells (33). Another similar miRNA with an onco-suppressor activity is let-7, which negatively regulates Ras and leads to apoptosis or cellular senescence (34). miRNAs functioning as oncogenes, such as miR-21, target the tumor suppressors, tropomyosin 1 (35) and programmed cell death 4, in breast cancer cells (36). The miR-17-92 cluster may be regarded as a family of oncogenes, directly targeting many genes implicated in apoptotic pathways. Therefore, miRNAs can act both as oncogenes and tumor suppressors, depending on the particular miRNA and cell type.

The implication of miRNAs in the etiology of schizophrenia and bipolar disorder, has been reported in several studies. In the study by Perkins *et al*, 16 miRNAs were identified as dysregulated in a post-mortem brain sample consisting of 13 individuals with schizophrenia and 2 individuals with schizoaffective disorder (37). A more recent study by Kim *et al*, confirmed the implication of miRNAs in the prefrontal cortex of individuals with schizophrenia and bipolar disorder (25). Finally, the differential expression of miRNAs has been reported in other neurodegenerative disorders, such as autism (38), in Parkinson's disease (39) and Alzheimer's disease (40).

One of the limitations of our study was the rather small sample size. It should also be noted that it is extremely difficult to find patients with this type of co-morbidity (schizophrenia and cancer). The level of expression of miRNAs was analyzed in blood and not in the brain, thus representing an indirect analysis of brain miRNA expression levels. Consequently, the data obtained in this study present a general genetic predisposition regarding the implication of miR-183 in the co-morbidity of schizophrenia and cancer. This is the first clinical study that analyzes the level of a large number of miRNAs in patients with both diseases, in order to examine this hypothesis and provide an explanation as to the low cancer risk in schizophrenic patients. Although, early clinical studies have confirmed the decrease cancer risk in schizophrenic sample patients (3) the relationship between miR-183 expression levels in the peripheral blood and the brain, in our study remains unclear.

As a conclusion, our data indicate that the overexpression of miR-183 may be associated with the presence of schizophrenia and the absence of a solid tumor, while the low expression of miR-183 may be linked with schizophrenia and the presence of a solid tumor. Our data provide a possible molecular explanation regarding the low cancer risk in patients suffering from schizophrenia. Further studies are warranted with a larger sample size, in order to establish the crucial role of miRNAs in the development of major diseases, such as schizophrenia and cancer and to elucidate the possible associations between them and the molecular pathways involved.

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