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Editorial Board

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Upregulation of miR-34a-3p and miR-744-3p is associated with downregulation of PTEN in lymphoblastoid cells from Shwachman-Diamond Syndrome patients

Jessica Gasparello[1], Chiara Papi[1], Giulia Breveglieri[1], Chiara Tupini[1], Elisabetta D'Aversa[1], Valentino Bezzerri[2], Marco Cipolli[2], Roberto Gambari[1], Monica Borgatti[1], Alessia Finotti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Cystic Fibrosis Center, Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona, Ancona, Italy
E-mail: alessia.finotti@unife.it

Shwachman-Diamond syndrome (SDS) is an inherited disease caused by mutations of a gene encoding for SBDS protein. SDS patients present several hematological disorders, including neutropenia and myelodysplastic syndrome (MDS), with increased risk of leukemic evolution. In lymphoblastoid cells of SDS patients, the already reported upregulated mTOR phosphorylation(1) is possibly associated with the downregulation of PTEN, a validated inhibitor of mTOR phosphorylation. Since PTEN expression might be under the post-transcriptional control of microRNAs(2), this study was undertaken to verify the miRNome in SDS patients and find a possible correlation with *PTEN* gene expression. Lymphoblastoid cell cultures derived from 7 SDS patients and 4 control subjects were studied by Next Generation Sequencing (NGS) and western blot analysis. In total, 11 miRNAs were upregulated in SDS cell cultures compared with controls, according to a 1.5-fold threshold. The differential miRNA expression was further validated by droplet-digital RT-PCR. When the 11 upregulated miRNAs were compared with the 81 validated PTEN-regulating miRNAs (identified using the miRTarBase software: *www. mirtarbase.mbc.nctu. edu.tw*), two microRNAs were identified: miR-34a-3p and miR-744-3p. In order to relate the PTEN expression with miR-34a-3p and miR-744-3p levels, PTEN was analyzed by RT-qPCR and western blot analysis. The results obtained supported the following conclusions: (a) upregulation of miR-744-3p and miR-34a-3p is variable among SDS cells; (b) PTEN downregulation was found in SDS cells to varying degrees; (c) a correlation does exist between the miR-34a-3p and miR-744-3p levels and *PTEN* gene expression. Consequently, miR-34a-3p and miR-744-3p are possible targets for increasing PTEN expression in SDS patients.

Acknowledgements: This study was supported by a FIR and a FAR fund of Ferrara University to AF, and by a fund from the Italian Ministry of Health (MOH) to VB, MB and GB.

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Analysis of the miRNome as a possible tool for the detection of autologous blood transfusion misuse in sport

Jessica Gasparello[1], Nicola Lamberti[2], Chiara Papi[1], Enrica Fabbri[1], Lucia Carmela Cosenza[1], Ilaria Lampronti[1], Fabio Manfredini[2], Roberto Gambari[1], Alessia Finotti[1]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara, Italy; [2]Department of Biomedical and Surgical Specialties Sciences - Section of Sport Sciences, Ferrara University, Ferrara, Italy; [3]Blood Transfusion Service Azienda Ospedaliera Universitaria Ferrara, Ferrara, Italy
E-mail: alessia.finotti@unife.it

Detection of Autologous Blood Transfusions (ABT) is a key issue in the field of anti-doping for the performance enhancing effects of this prohibited method and the consequent unfair use in sport. Unfortunately, at present no direct detection method of ABT is available. The present study was performed to determine whether the microarray-based analysis of the global miRNA pattern might be useful for the detection of ABT. Blood (500 ml) was drawn from 6 healthy subjects (T2) and then infused after 35 days (T5). Blood samples for microarray analysis of miRNAs were taken 5 days before (T1) and 10 days after blood withdrawal (T3), at day of infusion (T5), and at 3 days (T6) and 15 days (T8) after infusion. For three subjects the withdrawn blood was stored at -80°C, while for the remaining three at +4°C prior to infusion. Global microRNA profiling was performed for a total of 39 RNA samples (extracted from plasma), using the Agilent Human microRNA microarray v.21.0 (no. G4872A). This chip represents 2549 microRNAs, sourced from the miRBase database (Release 21). Microarray results were analyzed using the GeneSpring GX 13 software (Agilent Technologies). Differentially expressed miRNAs were selected following determination of the fold-change analysis, taking T1 as a reference sample. Both up- and downregulated miRNAs were considered. Microarray cluster analysis of informative miRNAs found in plasmas from ABT-trained athletes at T6 and T8 demonstrated that all the ABT T6 and T8 samples are clustered together and differ from T1 and from control samples. These results support the concept that the miRNome analysis might be considered a one-step approach for the detection of ABT.

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The possible low cancer risk in schizophrenic patients, through the regulatory role of microRNAs: Preliminary data

E. Rizos[1], N. Siafakas[2,1], E. Skourti[3], C. Papageorgiou[4], J. Tsoporis[5], Th. Parker[5], D.A. Spandidos[6], E. Katsantoni[7], K. Tsamakis[1], N. Nikolakakis[1,8], V. Zoumpourlis[3]
[1]National and Kapodistrian University of Athens, School of Medicine, 2nd Department of Psychiatry, University General Hospital "ATTIKON", Athens; [2]National and Kapodistrian University of Athens, Microbiology Laboratory, University General Hospital "ATTIKON", Athens; [3]Unit of Biomedical Applications, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, [4]National and Kapodistrian University of Athens, School of Medicine, 1st Department of Psychiatry, Eginition Hospital, Athens, Greece; [5]Keenan Research Centre, Li Ka Shing Knowledge Centre, Institute of Biomedical Science, St. Michael's Hospital, Toronto, Canada; [6]Laboratory of Clinical Virology, Medical School, University of Creta, Heraklion; [7]Biomedical Research Foundation, Academy of Athens, Hematology-Oncology Division, Athens; [8]Department of Psychiatry and Behavioral Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece
E-mail: erizos@med.uoa.gr

Interest has increased into the role of microRNAs (miRNAs) as regulatory factors in the aetiology and pathophysiology of major psychiatric disorders, such as schizophrenic spectrum disorder, bipolar disorder, depression etc. Reports have indicated a low cancer risk in patients with schizophrenic spectrum disorder and this risk seems to be related to the duration and age of onset of schizophrenia; specifically, duration seems to be inversely correlated with cancer risk. Moreover, other clinical reports have indicated the apoptotic activity of antipsychotics and mainly the First Generation Antipsychotics (FGAs) and thus, an impact on specific gene expression associated with the development of cancer. miRNAs are a large group of small non-coding oligonucleotides that regulate gene expression mainly in the Central Nervous System (CNS). While the development of cancer is characterized by increased gene expression that leads to uncontrolled cell proliferation, the development of schizophrenia is characterized by the opposite phenomenon and specifically, by the reduced expression of genes whose products suppress cellular proliferation and increase apoptosis. Therefore, the regulatory role of miRNA expression is crucial in the development of both of disorders and moreover, miRNAs emerge as a significant etiopathogenetic factors and possible biological markers for both disorders and may intermediate this possible negative correlation. This hypothesis is based on their pleiotropic and epistatic function; different subtypes can affect the expression of one gene and one type can act on many targets. In our previous study, we found an implication of let-7, mir-98 and mir-183 as possible biomarkers for cancer and schizophrenia (1,2). The scope of this review was to examine role of miRNAs in the rare co-morbidity of SZ and Ca and to highlight the possible molecular pathways which may be involved on both of these disorders (3). Moreover, new future medicinal strategies could be tested based on miRNA analysis, for both of the disorders, cancer and schizophrenic spectrum disorder.

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Monoclonal antibodies to human norovirus that was growing in cell culture

Vladimir Sologub, Irina Koromyslova
Research Center for Molecular Diagnostics and Therapy, Department of Biotechnology, Moscow, Russia

Monoclonal antibodies are the main tools for detection of antigens in a large variety of applications in the clinic and literature. Norovirus is a member of the Calicivirus family where many animal models with different biological but with the same morphological or physical properties were described. Replication in cell culture was shown for feline calicivirus and mouse norovirus, but attempts to cultivate human norovirus in cell culture were mainly unsuccessful. We have made successful attempt to cultivate Human Norovirus in monolayer cell culture. The cell culture was the high level of *in vitro* passage fibroblast-like human melanoma. The main property of the cells was their stability at 10 µg/ml of trypsin in serum-free media. Norovirus from fecal samples cause CPE in the cell monolayer after 3-4 days of cultivation at 37°C. Production of the virus was achieved by sandwich ELISA with pair of Mab's produced to Feline and Rabbit Caliciviruses and did not discriminate rabbit and feline viruses. Virus, at the level of 12th passage, was concentrated by ultracentrifugation and purified by $CsCl_2$ density gradient ultracentrifugation. Then, 100 µg of purified virus in 0.5 ml of PBS was mixed with Complete Friend's Adjuvant and injected into food patties of Balb/c mice. After 1 week, injection of purified virus was repeated and after 3 days popliteal lymph nodules were removed and the lymphocytes were fused with Sp/2-0 mouse myeloma. Hybridomas were detected by indirect ELISA with purified virus and gout anti-mouse IgG-HRP conjugate. In total, 29 clones were chosen and frozen in liquid nitrogen, and 12 Mab's were produced as ascetic fluids. The Mab's were purified by protein A chromatography and sandwich ELISA was used as well as HRP-conjugates of Mab's to generate common Calicivirus epitopes. Very sensitive detection of human norovirus and animal caliciviruses were then obtained by the panel.