Research Paper

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**MicroRNA expression profile in patients with cystic echinococcosis and identification of possible cellular pathways**

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**Abstract**

Cystic echinococcosis (CE) is a neglected tropical disease, caused by metacestode (larval) form of the *Echinococcus granulosus* sensu lato (sl) in humans. MicroRNAs (miRNAs) are small, stable, tissue-specific RNA molecules encoded by the genome that are not translated into proteins. Circulating miRNA expression profiles vary in health and disease. The aim of this study is to determine the altered cellular pathways in CE by comparing the miRNA profiles of controls and CE patients with active or inactive cysts. Following abdominal ultrasonography (US) examination, 20 patients diagnosed with active CE (CE1, CE2, CE3a and CE3b) or inactive CE (CE4 and CE5) and three healthy controls were included in the study. The expression profiles of 372 biologically relevant human miRNAs were investigated in serum samples from CE patients and healthy controls with miScript miRNA HCG PCR Array. Compared with the control group, expression of 6 miRNAs (hsa-miR-4659a-5p, hsa-miR-4518, hsa-miR-3977, hsa-miR-4692, hsa-miR-181b-3p, hsa-miR-4491) and one miRNA (hsa-miR-4687-5p) were found to be downregulated in CE patients with active and inactive cysts, respectively (*p* < 0.05). For downregulated miRNAs in this study, predicted targets were found to be associated mainly with cell proliferation, apoptosis, cell-cell interactions and cell cycle regulation. Further studies in this direction may elucidate the pathogenesis of human CE and the relationship between CE and other pathologies.

**Introduction**

Cystic echinococcosis (CE) is a neglected disease caused by metacestode (larval) form of the *Echinococcus granulosus* sensu lato (sl) in humans. CE is mostly endemic in rural areas of Australia, Asia, South America and Mediterranean countries (Deplazes *et al.*, 2017). Since it is mostly asymptomatic, definite prevalence of the disease is still unknown. However, the incidence of human CE is reported based on hospital records (Altintas, 2008). As reported in a recent study, CE prevalence is estimated to be approximately 0.61% in Turkey (Tamarozzi *et al.*, 2018). Although CE is mostly associated with a wide spectrum of symptoms, there is no specific finding. The hydatid cysts are mostly located in the liver and lung, with rates of about 70% and 25%, respectively (Akhan *et al.*, 1996; Brunetti *et al.*, 2018). Imaging techniques such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) are extensively used for diagnosis of CE. Serological tests have complementary role to imaging modalities in diagnosis. The World Health Organization-Informal Working Group on Echinococcosis (WHO-IWGE) has published a widely accepted classification of liver hydatid cyst based on the activity of the disease. Based on this classification, hydatid cysts are divided into three clinical groups as active (CE1 and CE2), transitional (CE3a/CE3b) and inactive (CE4 and CE5) (Brunetti *et al.*, 2010; Kern *et al.*, 2017). Although imaging techniques generally provide sufficient data for the diagnosis of CE, serological tests are needed in some cases. Unfortunately, there are considerable drawbacks in sensitivity/specificity (Se/Sp) and prognostic value of serological tests (Akhan & Ozmen, 1999; Manzano-Román *et al.*, 2015). While several studies are reported that cyst characteristics affected CE serology, there is still limited data available on underlying mechanisms of the host during CE development and progression.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, that have function in RNA silencing and post-transcriptional regulation of gene expression. Some miRNA families are predominantly expressed in certain tissues while some others are specific to certain biological processes (Negrini *et al.*, 2009; Li *et al.*, 2010). Unique circulating miRNA expression profiles have been demonstrated for various types of diseases. Mammalian miRNAs are
known to be stable in extracellular fluids such as plasma, serum, urine, saliva and semen (Mitchell et al., 2008; Olivieri et al., 2017). With the discovery of the disease-specific miRNAs in the blood of patients with cancer, metabolic disorders or viral infections, miRNA expression profiles have been widely studied particularly in infectious diseases that are difficult to diagnose and follow-up. (Tritten et al., 2014). Besides, miRNAs have a crucial role in the regulation of host–pathogen relation due to their function as post-transcriptional mechanism regulators (Cai et al., 2016).

The aims of this study are (1) to determine the alterations in miRNA expression profiles of patients with active and inactive cysts compared with healthy controls and (2) to identify altered cellular pathways in CE patients.

Materials and methods

Ethics statement

This study was approved by the Institutional Ethical Committee of the Faculty of Medicine (GO 17/711-16).

Sample collection, RNA extraction and cDNA synthesis

Twenty confirmed CE patients with 23 CE cyst (13 female and seven male) and three healthy controls (one female and two male, mean age ±40, without any underlying chronic and infectious disease) were included in this study. During US examination, the WHO-IWGE classification was used to evaluate the cases (Brunetti et al., 2010; Kern et al., 2017). Total RNA extraction was performed from serum of the patients/controls by miRNeasy Mini Kit (Qiagen) following the manufacturer’s instructions. Quality and purity of the RNA was verified by a NanoDrop 2000c instrument (ThermoScientific). For cDNA synthesis, miScript II RT system (Qiagen) were used according to manufacturer’s recommendations.

miRNA expression profiles and pathway analyses

miScript SYBR Green PCR Kit and miScript miRNA HC PCR Arrays (with a LightCycler 480 instrument II (Roche, Germany)) were used for the detection and quantification of miRNAs in serum. The miScript miRNA HC PCR Array provides expression profiles of 372 pathway/disease/functionally related mature miRNAs. Data analysis was performed using an online GeneGlobe data analysis centre (https://geneglobe.qiagen.com/no/analyze/), which uses the comparative CT (ΔΔCT) method for relative quantification and indicates fold change calculations. p < 0.05 was considered as statistically significant.

For miRNA target prediction miRDB (http://mirdb.org/), Targetscan (http://www.targetscan.org/vert_72/), and DIANA Tools (http://diana.imis.athena-innovation.gr/DianaTools/index.php) were used (Friedman et al., 2009; Paraskevopoulou et al., 2013; Liu & Wang, 2019; Chen & Wang, 2020).

Results

Demographics of patients and clinical characteristics

The majority of the cases were female (65%, 13/20). Mean age of the patients were 37.7 (range 7–69 years).

Table 1. Characteristics of patients.

| Number | Gender | Age  | Cyst number | Cyst type | Cyst location in liver | Cyst Size (cm) | Cyst volume (cm³) |
|--------|--------|------|-------------|-----------|------------------------|----------------|--------------------|
| 1      | Female | 24   | 1           | CE1       | Right lobe             | 5–10           | 110.26             |
| 2      | Female | 37   | 1           | CE1       | Left lobe              | 5–10           | 168.48             |
| 3      | Female | 25   | 1           | CE1       | Left lobe              | >10            | 884                |
| 4      | Female | 21   | 1           | CE1       | Right lobe             | >10            | 892.32             |
| 5      | Female | 46   | 1           | CE1       | Left lobe              | 5–10           | 278.46             |
| 6      | Female | 54   | 1           | CE2       | Right lobe             | >10            | 280.28             |
| 7      | Male   | 42   | 1           | CE2       | Right lobe             | 5–10           | 219.37             |
| 8      | Female | 30   | 1           | CE2       | Left lobe              | <5             | 65                 |
| 9      | Male   | 7    | 1           | CE2       | Left lobe              | 5–10           | 84.5               |
| 10     | Female | 18   | 2           | CE3b      | Right lobe             | >10            | 224.64             |
| 11     | Male   | 26   | 1           | CE3a      | Left lobe              | <5             | 62.4               |
| 12     | Male   | 30   | 1           | CE3b      | Right lobe             | 5–10           | 203.84             |
| 13     | Male   | 64   | 1           | CE4       | Right lobe             | 5–10           | 139.94             |
| 14     | Female | 33   | 2           | CE4       | Right lobe             | 5–10           | 178.36             |
| 15     | Female | 41   | 1           | CE4       | Right lobe             | 5–10           | 219.37             |
| 16     | Male   | 31   | 2           | CE4       | Right lobe             | <5             | 56.62              |
| 17     | Female | 52   | 1           | CE4       | Right lobe             | >10            | 484.38             |
| 18     | Male   | 69   | 1           | CE5       | Right lobe             | <5             | 53.76              |
| 19     | Female | 60   | 1           | CE5       | Right lobe             | <5             | 40.95              |
| 20     | Female | 44   | 1           | CE5       | Left lobe              | 5–10           | 374.86             |
Five of the 23 hydatid cysts were identified as CE1, four as CE2, one as CE3a, three as CE3b, seven as CE4 and three as CE5. Among 20 patients, 17 had a single cyst, the rest harboured two hydatid cysts. None of the patients had more than one cyst type. The mean size and volume of cysts were recorded as 7.4 cm and 251 cm³, respectively. All cysts were located in the liver (14/23 right lobe, 9/23 left lobe) (table 1). All the serum samples were positive for hydatidosis either by enzyme-linked immunosorbent assay (Hydatidosis IgG ELISA, Vircell SL, Granada, Spain) or indirect hemaggulination assay (Hydatidose, FUMOUZE Laboratories, France).

miRNA expression profiles

In patients with active cysts, a total of 6 miRNA (hsa-miR-4659a-5p, hsa-miR-4518, hsa-miR-3977, hsa-miR-4692, hsa-miR-181b-3p, hsa-miR-4491) and in inactive CE patients only one miRNA (hsa-miR-4687-5p) expression was found to be downregulated by at least twofold (p < 0.05) compared with healthy controls. Relative expression profiles of these miRNAs in active and inactive patients are presented in figs 1 and 2.

Pathway analysis

Downregulated miRNAs in active CE patients mainly regulate the expressions of cancer related oncogenes and tumour suppressor genes through cell proliferation, cell cycle, cell–cell interaction, transport systems, DNA repair and translational regulation. Additionally, these miRNAs can also play a role in neuronal growth process, function, differentiation and expression of immunoglobulin superfamilies.

Downregulated miRNA in inactive CE patients mainly regulates various cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation.

Discussion

Since the correlation between the expression of miRNAs in circulation and various pathologies has been determined, miRNAs are thought to be promising diagnostic biomarkers (Schwarzenbach et al., 2014). Although many studies focused on miRNA expression profiles in parasitic infections, there are only two studies conducted on the miRNA-based analysis of CE patients. In the study conducted by Mariconti et al., the expression profile of immune-related miRNAs was reported to be altered between active and inactive CE patients (Mariconti et al., 2019). According to their findings, six miRNAs in active CE patients were found to be upregulated compared with inactive CE patients. miRNAs detected in their study, have role in various immune-related processes such as proliferation/activation of macrophages, inflammation, apoptosis and/or oxidative damage, the regulation of the innate immunity, the type I interferon signalling and tumour suppression in many types of cancers (Mariconti et al., 2019). In the other study, parasite-derived miRNAs, egr-miR71 and egr-let 7, which were detected in circulation of CE patients found to be decreased after the removal of the cyst. These miRNAs are considered promising biomarkers for CE; however, the study does not provide any information about the pathways affected in the host in presence of CE (Alizadeh et al., 2020).

According to our results, compared with control group down-regulation in six miRNAs and one miRNA expressions were determined in active and inactive CE patients, respectively. These miRNAs were already known to be mainly involved in cell proliferation, apoptosis, cell–cell interactions and cell cycle. Current knowledge has suggested that miRNAs can play antitumoral or oncogenic roles in different cancer types. It is known that hsa-miR-4659a-5p, hsa-miR-4518, hsa-miR-181b-3p and hsa-miR-4687-5p, which were determined to be downregulated in this study, have a role in cancer development. In particular, an association between hsa-miR-4659a-5p and advanced breast cancer has been reported (Tabatabian et al., 2020). In a recent study, miR-4518 expression was found to be downregulated significantly in glioma tissues (Lu et al., 2018). miR-181b-3p was found to induce epithelial–mesenchymal transition which is crucial for increased invasion and metastasis during cancer progression in MCF7 breast cancer cells (Yoo et al., 2016). Additionally, miR-181b-3p was recently found as one of the miRNAs with potential in discriminating neck lymph node metastasis, suggesting that this miRNA has a potential as a prognostic biomarker (Liu et al., 2020). Lastly, miR-4687-5p was found to be upregulated in lung adenocarcinoma metastasis and considered to be among potential miRNA biomarkers for small cell lung cancer (Xu et al., 2020).

Some parasitic infections are well-known risk factors for various types of cancer in mammalian hosts. On the other hand, reports on the anticancer effects of parasitic organisms are limited. However, there are conflicting reports on the effect of E. granulosus on cancer risk. A retrospective study on CE
suggested a negative correlation between CE and solid tumours (Akgül et al., 2003). In contrast, Oikonomopoulou et al. proposed that infection by *E. granulosus* may increase the risk of cancer (Oikonomopoulou et al., 2016). Relationship between CE and cancer has not been clearly defined yet. Many studies also suggest a protective effect of *E. granulosus* and its products against cancer development. However, molecular mechanisms still need to be clarified (Guan et al., 2019).

To date, several mechanisms have been proposed to explain CE related anticancer effects, including parasite molecules and activation of host immune response. Ranasinghe et al. proposed that Kunitz-type protease inhibitor EgKI-1 which is highly expressed by the oncosphere of *E. granulosus* could disrupt the regular cell cycle and induce apoptosis in cancer cells (Ranasinghe et al., 2015, 2018).

In this study, most of the downregulated miRNAs in CE patients were found to be associated with cell proliferation, apoptosis, cell–cell interactions and cell cycle. Downregulation of these miRNAs due to the presence of the CE cyst possibly play a regulatory role in the anti-cancer effect.

This research has particular limitations. The patients in this study were limited to liver CE. Thus, further research including CE patients with other organ involvement like lungs, kidneys, etc., is needed to verify whether these miRNAs play an essential role in *E. granulosus* infection.

In conclusion, we showed that profiles of miRNA expression vary in active and inactive CE cysts of the liver. According to pathway analysis based on alterations in miRNA profiles, targets were predicted to involve mainly cell proliferation, apoptosis, cell–cell interactions and cell cycle control. miRNAs downregulated in CE should be considered as promising molecules in the mechanism based on the relation between cancer development and CE. Further studies based on host miRNAs are needed to confirm the affected genes in the presence of CE and to enlighten underlying molecular mechanisms of the relationship between CE and cancer.

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**Conflicts of interest.** None.

**References**

Akgül H, Tez M, Unal AE, Keşkek M, Sayık İ and Ozçelik T (2003) Echinococcus against cancer: Why not? Cancer 98, 1999–2000.

Akhan O and Ozmen MN (1999) Percutaneous treatment of liver hydatid cysts. European Journal of Radiology 32, 76–85.

Akhan O, Ozmen MN, Dinçer A, Sayık I and Göçmen A (1996) Liver hydatid disease: Long-term results of percutaneous treatment. Radiology 198, 259–264.

Alizadeh Z, Mahami-Oskouei M, Spotin A, Kazemi T, Ahmadpour E, Cai P, Shanehbandi D and Shekari N (2020) Parasite-derived microRNAs in plasma as novel promising biomarkers for the early detection of hydatid cyst infection and post-surgery follow-up. Acta Tropica 202, 105255.

Altintas N (2008) Parasitic zoonotic diseases in Turkey. Veterinaria Italiana 44, 633–646.

Brunetti E, Kern P and Vuitton DA (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Tropica 114, 1–16.

Brunetti E, Tamarozzi F, Macpherson C, et al. (2018) Ultrasound and cystic echinococcosis. Ultrasound International Open 4, E70–e78.

Cai P, Gobert GN and McManus DP (2016) MicroRNAs in parasitic helminthiases: Current Status and future perspectives. Trends in Parasitology 32, 71–86.

Chen Y and Wang X (2020) miRDB: An online database for prediction of functional microRNA targets. Nucleic Acids Research 48, D127–D131.

Deplazes P, Rinaldi L, Alvarez Rojas CA, et al. (2017) Global distribution of alveolar and cystic echinococcosis. Advances in Parasitology 95, 315–493.

Friedman RC, Farh KK, Burge CB and Bartel DP (2009) Most mammalian miRNAs are conserved targets of microRNAs. Genome Research 19, 92–105.

Guan W, Zhang X, Wang X, Lu S, Yin J and Zhang J (2019) Employing parasite against cancer: A lesson from the canine tapeworm echinococcus granulococcus. Frontiers in Pharmacology 10, 1137.

Kern P, Menezes da Silva A, Akhan O, Müllhaupt B, Vizcaychipi KA, Budke C and Vuitton DA (2017) The echinococcoses: Diagnosis, clinical management and burden of disease. Advances in Parasitology 96, 259–369.

Li M, Li J, Ding X, He M and Cheng SY (2010) microRNA and cancer. *The AAPS Journal* 12, 309–317.

Liu W and Wang X (2019) Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. *Genome Biology* 20, 18.
Liu KYP, Zhu SY, Brooks D, et al. (2020) Tumor microRNA profile and prognostic value for lymph node metastasis in oral squamous cell carcinoma patients. Oncotarget 11, 2204–2215.

Lu YF, Cai XL, Li ZZ, et al. (2018) LncRNA SNHG16 functions as an oncogene by sponging MiR-4518 and Up-regulating PRMT5 expression in glioma. Cellular Physiology and Biochemistry 45, 1975–1985.

Manzano-Román R, Sánchez-Ovejero C, Hernández-González A, Casulli A and Siles-Lucas M (2015) Serological diagnosis and follow-up of human cystic echinococcosis: A new hope for the future? Biomed Research International 2015, 428205.

Mariconti M, Vola A, Manciulli T, Genco F, Lissandrin R, Meroni V, Rosenzvit M, Tamarozzi F and Brunetti E (2019) Role of microRNAs in host defense against echinococcus granulosus infection: A preliminary assessment. Immunologic Research 67, 93–97.

Mitchell PS, Parkin RK, Kroh EM, et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America 105, 10513–10518.

Negrini M, Nicoloso MS and Calin GA (2009) MicroRNAs and cancer—new paradigms in molecular oncology. Current Opinion in Cell Biology 21, 470–479.

Oikonomopoulou K, Yu H, Wang Z, et al. (2016) Association between echinococcus granulosus infection and cancer risk - a pilot study in Cyprus. Clinical Chemistry and Laboratory Medicine 54, 1955–1961.

Olivieri F, Capri M, Bonafè M, Morsiani C, Jung HJ, Spazzafumo L, Viña J and Suh Y (2017) Circulating miRNAs and miRNA shuttles as biomarkers: Perspective trajectories of healthy and unhealthy aging. Mechanisms of Ageing and Development 165, 162–170.

Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M, Filippidis C, Dalamagas T and Hatziigiou AG (2013) DIANA-microT web server v5.0: Service integration into miRNA functional analysis workflows. Nucleic Acids Research 41, W169–W173.

Ranasinghe SL, Fischer K, Zhang W, Gobert GN and McManus DP (2015) Cloning and characterization of two potent Kunitz type protease inhibitors from echinococcus granulosus. PloS Neglected Tropical Diseases 9, e0004286.

Ranasinghe SL, Boyle GM, Fischer K, Potriquet J, Mulvenna JP and McManus DP (2018) Kunitz type protease inhibitor EgKI-1 from the canine tapeworm echinococcus granulosus as a promising therapeutic against breast cancer. PloS One 13, e0200433.

Schwarzenbach H, Nishida N, Calin GA and Pantel K (2014) Clinical relevance of circulating cell-free microRNAs in cancer. Nature Reviews Clinical Oncology 11, 145–156.

Tabatabian M, Mesrian Tanha H, Tabatabaee H, Sadeghi S, Ghaedi K and Mohamadynejad P (2020) Erbb4 3’-UTR variant (c."3622A > G) is associated with ER/PR negativity and advanced breast cancer. Indian Journal of Clinical Biochemistry 35, 115–120.

Tamarozzi F, Akhan O, Cretu CM, et al. (2018) Prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania, and Turkey: A cross-sectional, ultrasound-based, population study from the HERACLES project. The Lancet Infectious Diseases 18, 769–778.

Tritten L, Burkman E, Moorhead A, Satti M, Geary J, Mackenzie C and Geary T (2016) Detection of circulating parasite-derived microRNAs in filarial infections. PloS Neglected Tropical Diseases 8, e2971.

Xu Z, Wang Z, Sun H and Xin H (2020) Evaluation of exosomal miRNA in blood as a potential diagnostic biomarker for human Non-small cell lung cancer. Medical Science Monitor 26, e924721.

Yoo JO, Kwak SY, An HJ, Bae IH, Park MJ and Han YH (2016) miR-181b-3p promotes epithelial-mesenchymal transition in breast cancer cells through snail stabilization by directly targeting YWHAG. Biochimica et Biophysica Acta 1863, 1601–1611.