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Research article

Transcriptional over-expression of chloride intracellular channels 3 and 4 in malignant pleural mesothelioma

Vasiliki Tasiopoulou[a], Dimitrios Magouliotis[a], Evgeniy I. Solenov[b,c], Georgios Vavougios[d], Paschalis-Adam Molyvdas[a], Konstantinos I. Gourgoulianis[d], Chrissi Hatzoglou[a,d], Sotirios G. Zarogiannis[a,d,*]

[a] Department of Physiology, Faculty of Medicine, University of Thessaly, BIOPOLIS, Larissa 41500, Greece
[b] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk 630090, Russia
[c] Novosibirsk State University, Novosibirsk 630090, Russia
[d] Department of Respiratory Medicine, Faculty of Medicine, University of Thessaly, BIOPOLIS, Larissa 41500, Greece

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ABSTRACT

Background: Chloride Intracellular Channels (CLICs) are contributing to the regulation of multiple cellular functions. CLICs have been found over-expressed in several malignancies, and therefore they are currently considered as potential drug targets. The goal of our study was to assess the gene expression levels of the CLIC's 1–6 in malignant pleural mesothelioma (MPM) as compared to controls.
Methods: We used gene expression data from a publicly available microarray dataset comparing MPM versus healthy tissue in order to investigate the differential expression profile of CLIC 1-6. False discovery rates were calculated and the interactome of the significantly differentially expressed CLICs was constructed and Functional Enrichment Analysis for Gene Ontologies (FEAGO) was performed.
Results: In MPM, the gene expressions of CLIC3 and CLIC4 were significantly increased compared to controls ($p = 0.001$ and $p < 0.001$ respectively). A significant positive correlation between the gene expressions of CLIC3 and CLIC4 ($p = 0.0008$ and Pearson's $r = 0.51$) was found. Deming regression analysis provided an association equation between the CLIC3 and CLIC4 gene expressions: CLIC3 = 4.42CLIC4–10.07.
Conclusions: Our results indicate that CLIC3 and CLIC4 are over-expressed in human MPM. Moreover, their expressions correlate suggesting that they either share common gene expression inducers or that their products act synergistically. FAEGO showed that CLIC interactome might contribute to TGF beta signaling and water transport.

1. Introduction

Malignant pleura mesothelioma (MPM) is a rare and aggressive form of cancer that develops in the mesothelial pleural membrane that surrounds the lungs and chest wall. Exposure to asbestos is the main causative agent that leads to mesothelioma and therefore it is considered an occupational disease (Carbone et al., 2012). The incidence of malignant mesothelioma is expected to increase the next 10 years, while in industrialized Western nations currently nearly 8000 patients die due to MPM per year (Carbone et al., 2012). Although a lot of efforts have been underway aiming at identifying the best possible targets for novel therapies no actual progress has been made in prolonging the median survival of 1 year after diagnosis for MPM patients (Carbone et al., 2012). On the other hand it is very important to discover effective biomarkers for the early diagnosis of MPM and transcriptomic studies along with computational biology analysis seem to be a very powerful tool (Gordon et al., 2005; Jagirdar et al., 2013).

Over the last decade, ion channel biology has become an area of interest in oncology given that several ion channels have been shown to regulate specific stages of tumor progression and they have been implicated in several molecular mechanisms such as cell proliferation, invasion, cell circle regulation and metastatic spread (Prevarskaya et al., 2010). Moreover, deregulation of ion and water channels have been demonstrated in many primary human

* Corresponding author at: Department of Physiology, Faculty of Medicine, University of Thessaly, BIOPOLIS, 41500, Larissa, Greece. Fax: +30 2410 685555.
E-mail address: szarog@med.uth.gr (S.G. Zarogiannis).

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cancers and their blockade has shown tumor suppressing effects, both in vivo and in vitro (Prevarskaya et al., 2010).

Chloride intracellular channels (CLICs) are a family of ion channels that have been shown overexpressed in several malignancies. Although no data exist on the potential expression or role of CLIC members in MPM, it has been demonstrated that especially CLIC1 and CLIC4 have significant roles in cancer and more specifically in tumor metastasis, tumor aggressiveness, apoptosis evasion, cell proliferation and epithelial to mesenchymal transition (Peretti et al., 2015).

In this study we opted to assess the gene expression of CLICs in patients diagnosed with MPM as compared to healthy tissue and report that CLIC3 and CLIC4 are significantly overexpressed. Additionally, we found a linear association between the gene expression of the two and after constructing a network with their interactors we conducted a functional genomic analysis that revealed important roles of the CLIC3 and CLIC4 interactome that are pertinent to epithelial to mesenchymal transition as well as to water transporting properties. Our results provide a solid basis for future experiments in order to dissect the role of CLICs in MPM.

2. Materials and methods

2.1. Assessment of the differential gene expression of CLICs in MPM

We used data derived from the Oncomine Cancer Microarray database (http://www.oncomine.org/) in order to investigate the expression profiles of CLICs in MPM in comparison to healthy tissue that served as control. All analyses were performed during February of 2013. In order to compare the differential gene expression of CLIC genes in MPM ensuring that the data are adequate and were produced with the same methodology, we selected gene expression data from one study, the Gordon Mesothelioma study (Gordon et al., 2005). In this study samples from 40 MPM patients and 9 healthy samples (5 pleural and 4 lung derived) were assessed. The following relevant genes of the family were assesses in this study: CLIC1 (chloride intracellular channel 1), CLIC2 (chloride intracellular channel 2), CLIC3 (chloride intracellular channel 3), CLIC4 (chloride intracellular channel 4), CLIC5 (chloride intracellular channel 5), CLIC6 (chloride intracellular channel 6). CLIC6 data were not available in the study interrogated. The gene expression data were log transformed, median centered per array, and the standard deviation was normalized to one per array as described previously (Rhodes et al., 2004). Genes were considered to be over- or under- expressed when the fold change in a given group was significantly higher or lower compared to controls ($p < .05$). For further validation the false discovery rates (FDR; expressed as Q statistic) were calculated to further corroborate the validity of the results. The calculation of the Q statistic was based on the formula given by Rhodes and coworkers (Rhodes et al., 2004).

2.2. Construction of the gene interaction networks of the significantly differentially expressed CLICs

The gene interaction networks of significantly differentially expressed members of CLICs were constructed in String 9.1 software. String 9.1 is a database of known and predicted protein–protein interactions including both direct and indirect associations by quantitatively integrating interaction data from various sources (Franchescini et al., 2013). A Venn diagram was constructed using online tools in order to highlight common elements between the two lists. The goal of the network construction of significant CLICs was to extract their interactome in order to proceed to functional enrichment analysis of gene ontologies (GO).

2.3. Functional enrichment analysis of GO

The functional enrichment analysis of GO was performed with the use of the GeneMania software (Zuberi et al., 2013). GeneMania finds genes that are related to a set of input genes through a large set of functional association. However, its main strength is that it has an option to produce predictions of functional relationships between genes by mapping known relationships from other organisms via orthology.

2.4. Statistical analysis

The results were analyzed using GraphPad Prism 4.0 for Mac (GraphPad Software, San Diego, USA). Values that are negative reflect under-expression of a gene and values that are positive reflect over-expression of a gene. Normal distribution of the data was performed by application of the D'Agostino & Pearson omnibus normality test. Comparisons of gene expressions were performed with one-tailed Unpaired *t*-test for parametric data and with Mann–Whitney test for non-parametric data. Correlations between significantly differentially expressed genes were performed by the calculation of the Pearson (r) correlation coefficient. Deming regression analysis among the same genes was performed in order to assess cause-and-effect relationships among significant genes. This type of regression is used when experimental error is assumed for both X and Y variables. Differences were deemed significant with a p value less than 0.05.

3. Results

3.1. Transcriptional analysis of the differential gene expression of CLICs between normal subjects and MPM patients

Only two genes of the CLIC family were significantly over-expressed (Fig. 1), while the rest three genes were not differentially expressed between normal controls and MPM. As shown in Fig. 1A, the gene expression of CLIC3 was significantly increased compared to healthy controls ($p = 0.0007$). Similarly, the gene expression of CLIC4 ($p = 0.0003$; Fig. 1B) was significantly over-expressed in MPM. Calculation of the Q values further validated the strength of significance of our results (CLIC3, $q = 0.007$; CLIC4 $q = 0.019$). The gene expressions of CLIC1 ($p = 0.18$), CLIC2 ($p = 0.11$), and CLIC5 ($p = 0.40$) did not differ significantly between the two sample categories.

3.2. CLIC3 and CLIC4 gene expression is significantly correlated in MPM patients

The expression of CLIC3 was significantly correlated with CLIC4 MPM patients. More specifically, CLIC3 expression was found to correlate positively with CLIC4 expression ($p = 0.001$ and Pearson $r = 0.51$; Fig. 1C). Deming linear regression analysis provided cause-and-effect relationship between the expression of the two genes given by the following equation: *CLIC3 = 4.42 CLIC4-10.07*. In all other comparisons among CLIC genes no significant correlations occurred.

3.3. Construction of CLIC3 and CLIC4 interactome

The interrogation of the STRING 9.1 software revealed the 3 genes (DNM1, EZR and MAPK15) interacting with CLIC3 (Fig. 2A (i)) and 12 genes (DNM1, CO, ACTB, HRH3, SLC16A8, YWHAZ, HIVEP1, HIVEP2, SSTR2, PPM1A, BMPR2, SMAD2) interacting with CLIC4 (Fig. 2A (ii)). As shown in the Venn diagram in Fig. 2A (iii) there is only on element common, DNM1 (dynamin 1 gene) between the interactomes of CLIC3 and CLIC4.