EXPRESSION OF CIRCULAR RNA hSA_CIRC_0067705 IN MALIGNANT VERSUS NON MALIGNANT PLEURAL EFFUSION IN A COHORT OF EGYPTIAN PATIENTS

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Introduction

Malignant pleural effusion (MPE) is a frequent complication of many malignancies, with lung cancer being the most frequent etiology. Non-small cell lung cancer (NSCLC) is the most prevalent type and accounts for 85% of lung cancer cases. The presence of MPE in NSCLC patients denotes an end-stage disease with reduced overall survival. Pleural biopsy remains the gold standard diagnostic tool. However, it's considered invasive. Consequently, it's important to identify a pleural fluid biomarker for NSCLC diagnosis. Epigenetic disruptions were linked to NSCLC via oncogene activation or tumor suppressor gene silencing. Circular RNAs (circRNAs) have emerged as a class of non-coding RNAs (ncRNAs), which are known as epigenetic modifiers. CircRNAs are covalently closed loop structure confers them the resistance to Rnases compared to linear RNAs. They are abundantly expressed, with tissue specific patterns. They play role in tumor progression through different regulatory mechanisms such as miRNAs sponging activity and circRNA protein interactions. hsa_circ_0067705 a novel circRNA was found to be up regulated in NSCLC-MPE patients. Thus, it might be involved in NSCLC progression, however, the detailed mechanism and the expression pattern in NSCLC-MPE remain elusive.

Aim of the work

The aim of this study was to compare the expression level of circular RNA hsa_circ_0067705 in malignant pleural effusion (MPE) and non-malignant pleural effusion (non-MPE) in a cohort of Egyptian patients.

Patients and Methods

The study was conducted on 50 patients divided into two groups:

Group I: 25 patients with MPE recently diagnosed with NSCLC- MPE, by demonstration of malignant cells in pleural fluid and/or on a pleural biopsy sample.

Group II: 25 diagnosed patients with non-MPE of a matching age and sex as a comparable group.

All patients included in the present study were subjected to the following:

1.Complete history taking. 2.Complete physical examination. 3.Radiological investigations.

3. Laboratory Investigations including:

•Routine lab investigations:

- Complete blood count (CBC). - Renal function tests (serum urea and creatinine)
- Liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin and serum total protein

• Pleural fluid examination (physical, chemical and microscopic).

•Histopathological examination

•Molecular analysis: Pleural fluid samples were subjected to total RNA extraction, complementary DNA (cDNA) synthesis and relative quantification of hsa_circ_0067705 expression using quantitative real time polymerase chain reaction (qRT-PCR).

Results

hsa_circ_0067705 expression was significantly elevated in NSCLC-MPE patients when compared to non-MPE patients (p = 0.018).

Table 1: Comparison between the two studied groups according to expression of circular RNA hsa_circ_0067705 (fold change)

Expression of circular RNA hsa_circ_0067705 (fold change)	NSCLC-MPE (n = 25)	Non-MPE (n = 25)	U	p
Min. – Max.	0.01 – 130.65	0.01 – 58.47	190.5*	0.018*
Median (IQR)	8.22 (1.61 – 39.66)	1.14 (0.16 – 7.51)		

IQR: Inter quartile range U: Mann Whitney test

p: p value for comparing between the two studied groups *: Statistically significant at $p \leq 0.05$

Regarding hsa_circ_0067705 expression, there was a significant difference between NSCLC-MPE patients and patients with tuberculous effusion (p = 0.009). In addition, there was significant difference between NSCLC-MPE patients, patients with tuberculous effusion and patients with non-tubelcuous effusion (p = 0.027). There was no significant difference between non-tubelcuous effusion and NSCLC-MPE patients (p = 0.156).

Table 2: Comparison between the three studied subgroups according to expression of circular RNA hsa_circ_0067705 (fold change)

Expression of circular RNA hsa_circ_0067705 (fold change)	NSCLC-MPE (n = 25)	Non-MPE (n = 25)		H	p
		Tuberculosis (n = 10)	Non Tuberculosis (n = 15)		
Min. – Max.	0.01 – 130.7	0.04 – 28.44	0.01 – 58.47		
Median (IQR)	8.22 (1.61 – 39.66)	0.37 (0.12 – 1.21)	4.63 (0.22 – 10.82)	7.207*	0.027*
Significance between subgroups	p_1= 0.009*, p_2= 0.156, p_3= 0.206				

IQR: Inter quartile range SD: Standard deviation H: H for Kruskal Wallis test,

Pairwise comparison between each 2 groups was performat using Post Hoc Test (Dunn's for multiple comparisons test)

p: p value for comparing between the three studied subgroups p_1: p value for comparing between cases and tuberculosis

p_2: p value for comparing between cases and non-tuberculosis

p_3: p value for comparing between tuberculosis and non-tuberculosis *: Statistically significant at $p \leq 0.05$

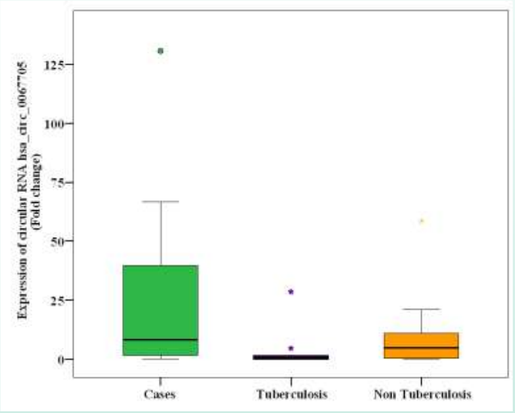

Figure: Comparison between the three studied groups according to expression of hsa_circ_0067705 (fold change). The upper and lower borders of the box represent the 25th and 75th percentiles. The horizontal line in the box represents the median (50th). Circles represent the outliers. Data were analyzed using kruskal Wallis test. Statistically significant at $p < 0.05$.

As for serum lactate dehydrogenase (LDH), there was a statistically significant difference between NSCLC-MPE and non-MPE patients (p = 0.032). In addition, a positive correlation was found between hsa_circ_0067705 expression and serum LDH in NSCLC-MPE (r_s = 0.401, p = 0.047).

Conclusion

According to the current study results it was concluded that:

- hsa_circ_0067705 is overexpressed in NSCLC-MPE patients than non-MPE patients and the expression level was positively correlated with serum LDH.
- hsa_circ_0067705could be a potential biomarker for the diagnosis of NSCLC-MPE and differentiate NSCLC-MPE from tuberculous effusion.

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