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Value of long chain non-coding rnacrnde-h and gas5 in predicting the prognosis of multiple myeloma.

Feixian Li, Qiying Wu*, Xinzhi Li, You Zhou, Tianbo Zhu, Qingbin Han

Department of Orthopedics, Affiliated Renhe Hospital of Three Gorges University, PR China

Abstract

Background: Multiple myeloma (MM) is a bone marrow malignant plasma cell disease characterized by focal bone lesions, soft tissue lesions and diffuses bone marrow infiltration. The purpose of this study is to observe and analyze the value of serum lncRNACRNDE - H and GAS5 in predicting the prognosis of MM. Methods: 80 cases of MM patients admitted from our hospital from December 2009 to December 2010 were selected for this study. All MM patients enrolled were treated with 4 courses of PAD program induction therapy. The follow-up was conducted for 4 years by telephone follow-up and follow-up visit patients, and the overall survival (OS) and progression free survival (PFS) of the patients were observed and compared. Results: The difference between OS and PFS in the two subgroups was statistically significant (χ^2=7.876-7.009, P<0.05). For patients with GAS5 low-expression and GAS5 high-expression, the OS estimated values were 34.030 months and 23.546 months respectively, 95 CI of (28.717-39.343) months and (19.270-27.823) month, PFS estimated values of 26.794 months and 19.753 montsh, 95% CI of (22.255-31.333) months and (15.897-23.609) months. Conclusion: High expression of serum lncRNACRNDE-h is associated with poor prognosis in patients with MM. The level of serum lncRNACRNDE-h can be used as an auxiliary marker to predict the prognosis of patients with MM.

Keywords: Long chain non-coding rnacrnde-h, GAS5, Multiple myeloma.

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Introduction

Multiple myeloma (MM) is a bone marrow malignant plasma cell disease characterized by focal bone lesions, soft tissue lesions and diffuses bone marrow infiltration. And MM ranks second in malignant hematologic diseases, mostly in middle aged and over 40 years old patients, and the median survival time is less than 1 years. In recent years, the incidence rate has risen gradually, but with the advent of newer and more effective treatments, the 5 year survival rate has increased from 25% in 1975 to 34% in 2003. The main lesion of MM patients is the proliferation of abnormal plasma cells, and the monoclonal immunoglobulin produced by this plasma cell have no immune function, thus the pathological changes such as the decrease of the immune function and the destruction of bone were found in the patients, which resulted in complex clinical manifestations of bone pain, anemia, renal dysfunction, infection, multisystem and multiple organ damage [1]. Although the elderly population is MM multiple population, but in recent years, the age of onset showed a trend of younger, and the lack of specific symptoms of MM can easily lead to misdiagnosis or missed diagnosis, and then the therapeutic effect and prognosis will be affected. Chemotherapy is the main method of clinical treatment of MM, but chemotherapy for refractory, recurrent MM patients has poor efficacy without significant survival extension [2]. Therefore, clinical research has recently developed new drugs such as lenalidomide, bortezomib and carbofi millet, and new treatments including sequential autologous hematopoietic stem cell transplantation (AHscT) and allogeneic hematopoietic stem cell transplantation (allo-HSCT). The results of these studies indicated that these new therapies had a certain value in improving the remission rate of MM patients and life quality [3]. However, MM patients have individual different response to drug, leading to the great difference of prognosis. Timely and accurate evaluation of the prognosis of patients with MM is of positive significance for the formulation of a reasonable treatment plan. With the continuous progress of medical science and technology, the studies for the diagnosis of MM, disease evaluation and prognosis are no longer limited to bone marrow biopsy and imageological examination, but to the biology directions of serum free light chain, cytogenetics and molecular biological detection [4]. Recent studies have shown that long-chain non-coding RNA (1ncRNA) played an important role in regulating the gene expression of normal cells and tumor cells. It can be involved in the proliferation, invasion and metastasis of tumor cells through the role of oncogene or tumor suppressor gene. Therefore, the researchers began to employ lncRNA as a marker for the prognosis of malignant tumors [5]. The purpose of this study is to observe and analyze the value of serum lncRNA ColoRectal Neoplasia Differentially Expressed (CRNDE) - H and growth arrest-specific 5 (GAS5) in predicting the prognosis of MM.

Materials and Methods

General data

A total of 80 cases of MM patients admitted from our hospital from December 2009 to December 2010 were selected for this study. All patients were in accordance with the MM diagnostic criteria of the international Multiple Myeloma Working Group (IMWG) and the WHO 2003/(WHO) 2008. The expected survival of the patients was over 3 months, and the patients were excluded from other malignant tumors, mental disorders and organic diseases of important organs. Among the enrolled patients, 42 were male and 38 were female, aged from 35 to 73 years old, with an average of (56.3 ± 8.1) years old. 80 cases of healthy people were selected as the control group, who were confirmed by clinical examination to exclude MM and other malignant tumors, among them, male were 45 cases, female of 35 cases, aged 37 to 76 years old, the average (57.6 ± 8.7) years old. There were no significant differences in age and gender between the two groups (P>0.05). All the subjects have signed informed consent, and the research program has been approved by the medical ethics committee of our hospital.

Therapeutic method

All MM patients enrolled were treated with 4 courses of PAD program induction therapy. The specific program was 1.75 mgdl of bortezomib, 20 mgdl of 4 epirubicin at d4, d8, dll, 20 mgdl of dexamethasone at dl-4 and d8- 11, 28 days for 1 course.

Observation index and detection method

Early morning fasting peripheral blood samples were collected from the two groups of subjects (before treatment). The serum was extracted and stored in a refrigerator at -80°C. The total RNA was extracted by miRNeasySerum/Plasma Kit. The lncRNACRNDE-h and GAS5 levels of serum samples were detected with real-time fluorescence quantitative PCR (Q-RTPCR) method, cel-miR-39 as the internal reference. The primer sequences were listed in Table 1, the reaction system was in Table 2 with a total of 45 cycles. The cycle thresholds (Ct) of target lncRNA and the internal reference, Ct was the difference between the Ct values of target lncRNA and cel-miR-39, and $2^{-\Delta\Delta ct}$ indicates the expression level of target lncRNA. According to the evaluation criteria of curative effect formulated by IMWG, the curative effect of 2 courses and 4 courses was evaluated and compared, which was mainly divided into complete remission (CR), partial remission (PR), stable disease (SD) and disease progression (PD), CR or PR was considered as clinical effectiveness; The follow-up was conducted for 4 years by telephone follow-up and follow-up visit patients, and the overall survival (OS) and progression free survival (PFS) of the patients were observed and compared.

Table 1. *The primer sequences of target IncRNA.*

Name	sequence	
CENDE-h	upstream primer	GCGGAGGAGAGGTGTTAAGTGT
	downstream primer	AACAGGTTTTACCTCCTTATCTTCAGAA
GASS	upstream primer	CTTGCCTGGACCAGCTTAAT
	downstream primer	CAAGCCGACTCTCCATACCT

Table 2. *The reaction system of q-RT PCR.*

Reagents	Volume
SYBR Premix Ex Taq II	5 μl
PCR Forward Primer (10 μM)	1 μl
PCR Reverse Primer (10 μM)	1 μl
cDNA template	1 μl
dH_2O (sterile purified water)	2 μl
total volume	10 μl

Statistical methods

All the data were analyzed by SPSS13.0. The measurement data were compared with the independent sample t test. The enumeration data were processed by chi-square test. The comparison of OS and PFS in patients with different serum lncRNA employed Kaplan-Meier Survival analysis, LogRank test detected survival difference, the Cox regression was used for multivariate analysis of risks affecting the survival, P<0.05 means the difference with statistically significance.

Results

Comparison of relative expression levels of serum IncRNACRNDE-h and GAS5 in two groups of subjects

The relative expression of serum lncRNACRNDE - H and GAs5 in the case group was higher than that in the control group, and the difference between the two groups was statistically significant, as shown in Table 3.

Table 3. *Comparison of relative expression levels of serum incRNACRNDE-h and GAS5 in two groups of subjects.*

Groups	Number of cases	lncRNA relative expression	
		CRNDE-h	GASS
Case group	80	0.572 ± 0.276	0.824 ± 0.361
Control group	80	0.307 ± 0.225	0.289 ± 0.175
T		5.658	6.295

P	<0.05	<0.05

Comparison of clinical efficacy in MM patients with different serum levels of lncRNA, CRNDE-h and GAS5 relative expression

The mean relative expression of serum lncRNA CRNDE-h was considered as the boundary value, the patients were divided into CRNDE-h low-expression group (40 cases) and CRNDE-h high-expression group (40 cases). The patients were divided into GAS5 low-expression group (40 cases) and GAS5 high-expression (40 cases) based on the mean relative expression of serum lncRNA GAS5. There were no statistically significant differences in the efficacy and clinical efficacy of MM in patients with different serum levels of lncRNA, CRNDE, h, and GAS5, as shown in Tables 4-7.

Table 4. *Comparison of the therapeutic effects of MM patients with different serum IneRNA CRNDE-h relative expression levels during the 2 course of treatment (cases, %).*

Group	Number of cases	Curative effect				Clinical efficacy rate
		CR	PR	SD	PD	
CRNDE-h-low expression	40	10 (25.0)	22 (55.0)	3 (7.5)	5 (12.5)	32 (80.0)
CRNDE-h-high expression	40	11 (27.5)	23 (57.5)	3 (7.5)	3 (7.5)	34 (85.0)
χ^2			0.570			0.346
P			0.903			0.556

Table 5. *Comparison of the therapeutic effects of MM patients with different serum IneRNA CRNDE-h relative expression levels during the 4 course of treatment (cases, %).*

Group	Number of cases	Curative effect				Clinical efficacy rate
		CR	PR	SD	PD	
CRNDE-h-low expression	40	17 (42.5)	18 (45.0)	3 (7.5)	2 (5.0)	35 (87.05)
CRNDE-h-high expression	40	19 (47.5)	17 (42.5)	3 (5.0)	2 (5.0)	36 (90.0)
χ^2			0.340			0.125
P			0.952			0.723

Table 6. *Comparison of the therapeutic effects of MM patients with different serum IneRNA GAS5 relative expression levels during the 2 course of treatment (cases, %).*

Group	Number of cases	Curative effect				clinical efficacy rate
		CR	PR	SD	PD	
GAS5 low expression	40	18 (45.0)	16 (40.0)	4 (10.0)	5 (12.5)	31 (77.5)
GAS5 high expression	40	18 (45.0)	19 (47.5)	2 (5.0)	3 (7.5)	35 (87.5)
U/χ^2						1.385
P						0.239

Table 7. *Comparison of the therapeutic effects of MM patients with different serum IneRNA GAS5 relative expression levels during the 4 course of treatment (cases, %).*

Group	Number of cases	Curative effect				Clinical efficacy rate
		CR		SD	PD	
GAS5 low expression	40	18 (45.0)	16 (40.0)	4 (10.0)	2 (5.0)	34 (85.0)
GAS5 high expression	40	18 (45.0)	19 (47.5)	1 (2.5)	2 (5.0)	35 (92.5)
U/χ^2						1.127
P						0.288

Comparison of prognosis in MM patients with different serum levels of lncRNA CRNDE-h and GAS5 relative expression

The follow-up period of this group was ended on December 31, 2014. Kaplan-Meier survival analysis showed that OS estimated value of MM patients in the group was 31.164 months, 95CI was (27.089-35.239) months, PFS estimated value was 24.835 months, 95% CI was (21.422-28.249) months. For patients with CRNDE-h low-expression and CRNDE-h high-expression, the OS estimated values were 37.125 months and 22.575 months respectively, 95 CI of (31.955-42.295) months and (18.589-26.561) months, PFS estimated of 29.538 months and 18.237 months (P<0.05), 95% CI of (25.213-33.864) months and (14.224-22.250) months. The difference between OS and PFS in the two subgroups was statistically significant (χ^2=7.876, 7.009, P<0.05). For patients with GAS5 low-expression and GAS5h high-expression, the OS estimated values were 34.030 months and 23.546 months respectively, 95 CI of (28.717-39.343) months and (19.270-27.823) months, PFS estimated values of 26.794 months and 19.753 months, 95% CI of (22.255-31.333) months and (15.897-23.609) months. There was no significant difference in OS and PFS between the two subgroups (χ^2=2.390, 1.444, P>0.05). Cox multiple regression analysis showed that OS was associated with thrombocytopenia (χ^2=5.125, P<0.05), lactate dehydrogenase (χ^2=4.875, P<0.05), P53 deletion (χ^2=5.824, P<0.05) and CRNDE-h overexpression (χ^2=6.091, P<0.05), PFS was related to P53 deletion (χ^2=6.545,

P<0.05), CRNDE-h overexpression (χ^2=7.881, P<0.05) (Table 8 and 9).

Table 8. Cox multivariate analysis of the overall survival (OS) of the group.

Variants	HR	Wald χ^2	P
thrombocytopenia	1.725	5.125	<0.05
lactate dehydrogenase-elevating	1.816	4.875	<0.05
P53 deletion	2.067	5.824	<0.05
CRNDE-h overexpression	1.956	6.091	<0.05

Table 9. Cox multivariate analysis of the progression free survival (PFS) of the group.

Variants	HR	Wald χ^2	P
P53 deletion	2.362	6.545	<0.05
CRNDE-h overexpression	2.033	7.881	<0.05

Discussion

At present, about 10, 000 kinds of lncRNA in the human genes have been identified [6,7]. Related studies have proved that some lncRNA were in high expression in malignant tumor tissue and serum of patients, involved in the proliferation, apoptosis, invasion, and metastasis process of tumor cells, and closely related to occurrence, recurrence, metastasis and resistance [8,9]. The present study has confirmed that the mechanism of lncRNA in malignant tumor pathological process included the effects on expression of wIF1, PTEN and p21, regulation of Wnt, Akt and p53 signal pathways, enhanced expression of E - cadherin and CDH13, decreased expression of Vim-entin, MMP2 and MMP9 and effect on cell cycle [10,11]. As a result, lncRNA is used as a specific marker for the diagnosis, targeted therapy, therapeutic evaluation and prognosis prediction of malignant tumor.

CRNDE is a gene symbol of ColoRectal Neoplasia Differentially Expressed, which can be transcribed into a multifunctional lncRNA expressed in specific regions of the human and mouse brain, showing a high expression in mice homologous inducing pluripotent stem cells and further increased in neuronal differentiation. CRNDE has different splice forms of polycomb inhibition complex 2 (PRC2), CoREST chromatin modification complex, which provides specific functional scaffolds for regulatory complexes and forms a very tissue-specific expression pattern. LncRNA CRNDE is closely related to t cancer, neurobiology and development. It was originally identified as a lncRNA high-expressed in colorectal cancer, but with the continuous expansion of research, expression of lncRNA is also elevated in many other solid tumors and leukemias. The complex selective splicing of CRNDE led to a large number of transcripts from the gene. While in vitro test for cancer cells, the researchers found that inhibition of CRNDE nuclear transcripts such as GVC-IN4 can inhibit PI3K/Akt/MTOR pathway or Raf/MAPK pathway, and thus affect the expression of many genes related to insulin/IGF and other related genes [13]. GAS5 is a genetic symbol of Growth Arrest-specifictranscript5, which encodes multiple ncRNAs, and its exon sequence transcribes lncRNA [14], and lncRNAGAS5 plays a role in inhibition of glucocorticoid and its receptors, negatively regulating the survival of lymph and mammary gland cells [15]. The current study has demonstrated that lncRNA GAS5 is associated with the occurrence and progression of a variety of malignancies. For example, lncRNAGAS5 can effectively regulate the pro-apoptotic activity of prostate cancer cells, and low levels of GAS5 can reduce the ability of prostate cells to chemotherapeutic agents reactivity [16]. Related research has shown that lncRNAGAS5 has a relation to the prognosis of renal cell carcinoma, non-small cell lung cancer (NSCLC) and other malignant tumors [17], therefore, it has also been the new molecular marker associated with malignant tumors. The results of this study showed that the relative expression of CRNDE-h and GAS5 in serum of patients was higher than that in the control group. There was no significant difference in the efficacy and clinical efficacy of patients with different serum lncRNA CRNDE-h and GAS5 relative expression, suggestting that MM patients can have high expression of serum lncRNA CRNDE-h and GAS5, while the two indicators can prompt the risk of MM, and used as serum markers of MM diagnosis, while there was a lack of correlation with the therapeutic efficacy of MM and the two indicators. The OS and PFS in patients with low expression of serum lncRNA CRNDE-h were significantly longer than those of high expression of serum lncRNA CRNDE-h, while the difference was not statistically significant. The OS were correlated to thrombocytopenia, elevating lactate dehydrogenase, P53 deletion and CRNDE-h overexpression, while PFS was associated with P53 deletion, CRNDE-h high expression, indicating that serum 1n-cRNA CRNDE-h high expression were related to poor prognosis of MM patients, serum lncRNA and CRNDE-h could be used as an adjunct indicator to predict the prognosis of MM patients.

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***Correspondence to**

Qiying Wu

Department of Orthopedics

Affiliated Renhe Hospital of Three Gorges University

PR China