

Maria Radanova^{1,2*}, Galya Mihaylova¹, Oskan Tasinov¹, Desislava Ivanova¹, Neshe Nazifova-Tasinova¹, Rostislav Manev^{1,2}, Ayshe Salim¹, Miglena Todorova¹, Diana Ivanova¹, Nikolay Conev^{1,2}, Zhasmina Mihaylova³, Ivan Donev⁴

1 Medical University of Varna, Varna, Bulgaria; 2 University Hospital "St. Marina", Varna, Bulgaria; 3 Military Medical Academy, Sofia Bulgaria; 4 Hospital Nadezhda, Sofia, Bulgaria

*corresponding author: maria.radanova@gmail.com

OBJECTIVES

Circular RNAs (circRNAs) are group of special endogenous long non-coding RNAs (ncRNAs). Their circular form makes them resistant to exonuclease activity compared to linear ncRNAs and thus more suitable as new biomarkers.

AIM

The aim of our study was to evaluate the expressions of two circRNAs – hsa_circ_0001445 and hsa_circ_0007915 in patients with colorectal cancer (CRC) in different stages of disease in comparison with healthy volunteers.

METHODS

122 patients with metastatic CRC, 29 adjuvant patients with stage III disease who had undergone radical surgery and postoperative chemotherapy and 90 healthy volunteers were investigated. All adjuvant patients had normal levels of CEA and they were followed-up regularly for 1 year after the end of their treatment and had no signs of radiological or biochemical relapse. CircRNAs were extracted from patients' plasma obtained prior to chemotherapy using a commercial kit. The circRNAs expressions in plasma were measured by qPCR and the relative expression was calculated applying $2^{-\Delta\Delta Ct}$ method.

RESULTS

Patients with metastatic CRC had significantly higher expression of hsa_circ_0001445 and hsa_circ_0007915 than adjuvant patients and healthy volunteers

At the optimal cut-off values for their plasma levels, hsa_circ_0001445 and hsa_circ_0007915 could significantly distinguish between patients with or without metastatic disease with 92.56% sensitivity and 42.86% specificity and with 86.07% sensitivity and 57.14% specificity (AUC=0.729, 95% CI: 0.62-0.84, p=0.0002; AUC=0.714, 95% CI: 0.59-0.84, p=0.0004), respectively.

The mean overall survival (OS) of patients with high/intermediate expression of hsa_circ_0001445 was 30 months (95% CI: 24.99-34.73) in comparison with the mean OS of the patients with low expression – 20 months (95% CI: 16.70-23.76) (log-rank test, p=0.034). In multivariate Cox regression analysis the low levels of hsa_circ_0001445 were also associated with shorter survival (HR=1.59, 95% CI: 1.02-2.47, p=0.040).

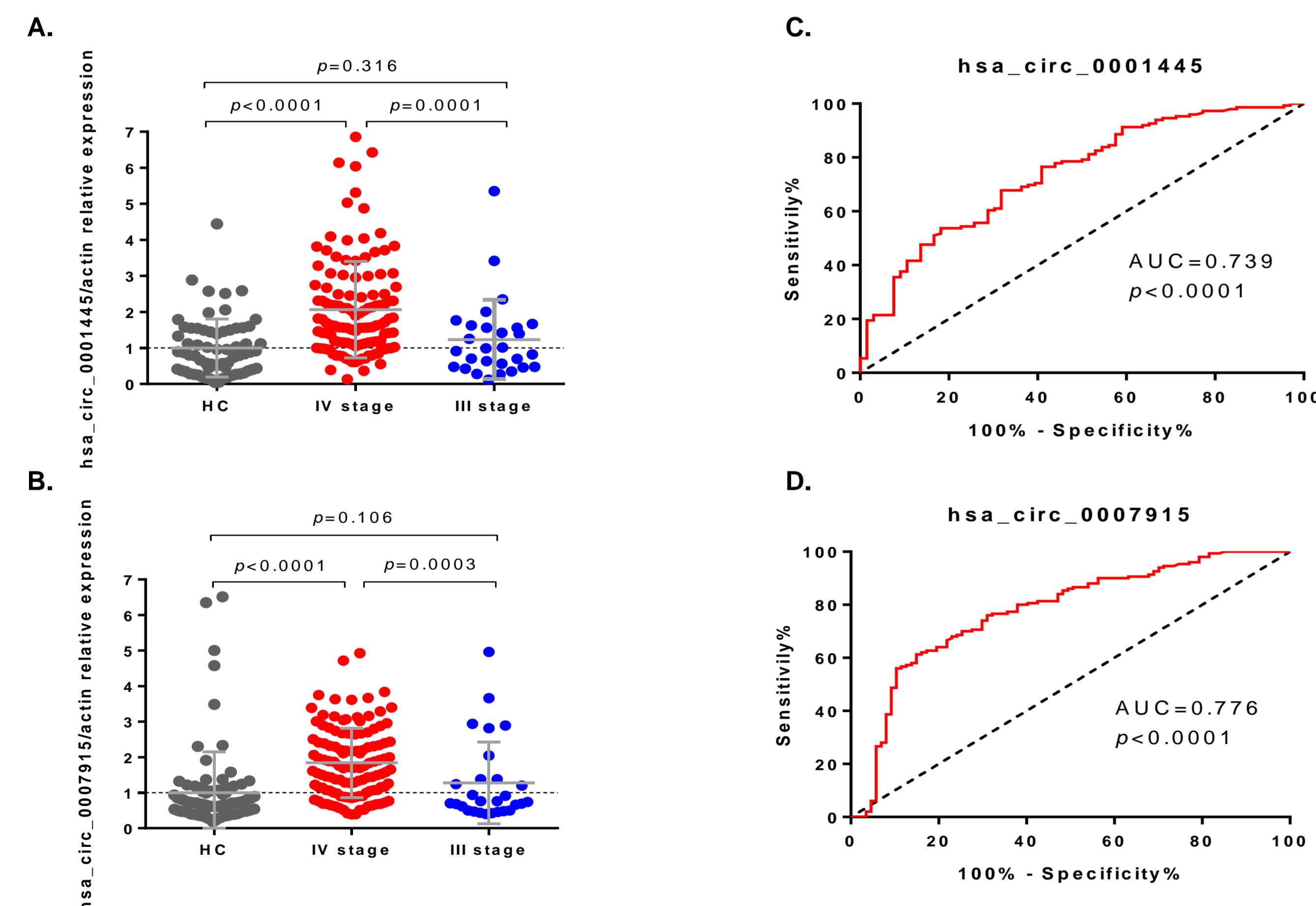


Figure 1. Comparison between levels of hsa_circ_0001445 (A.) and hsa_circ_0007915 (B.) in plasma samples from healthy controls (HC) and patients with CRC in IV and III stages (Mann-Whitney U test, data are presented as mean \pm SD). ROC curves of using hsa_circ_0001445 (C.), hsa_circ_0007915 (D.) to differentiate patients with CRC from HC.

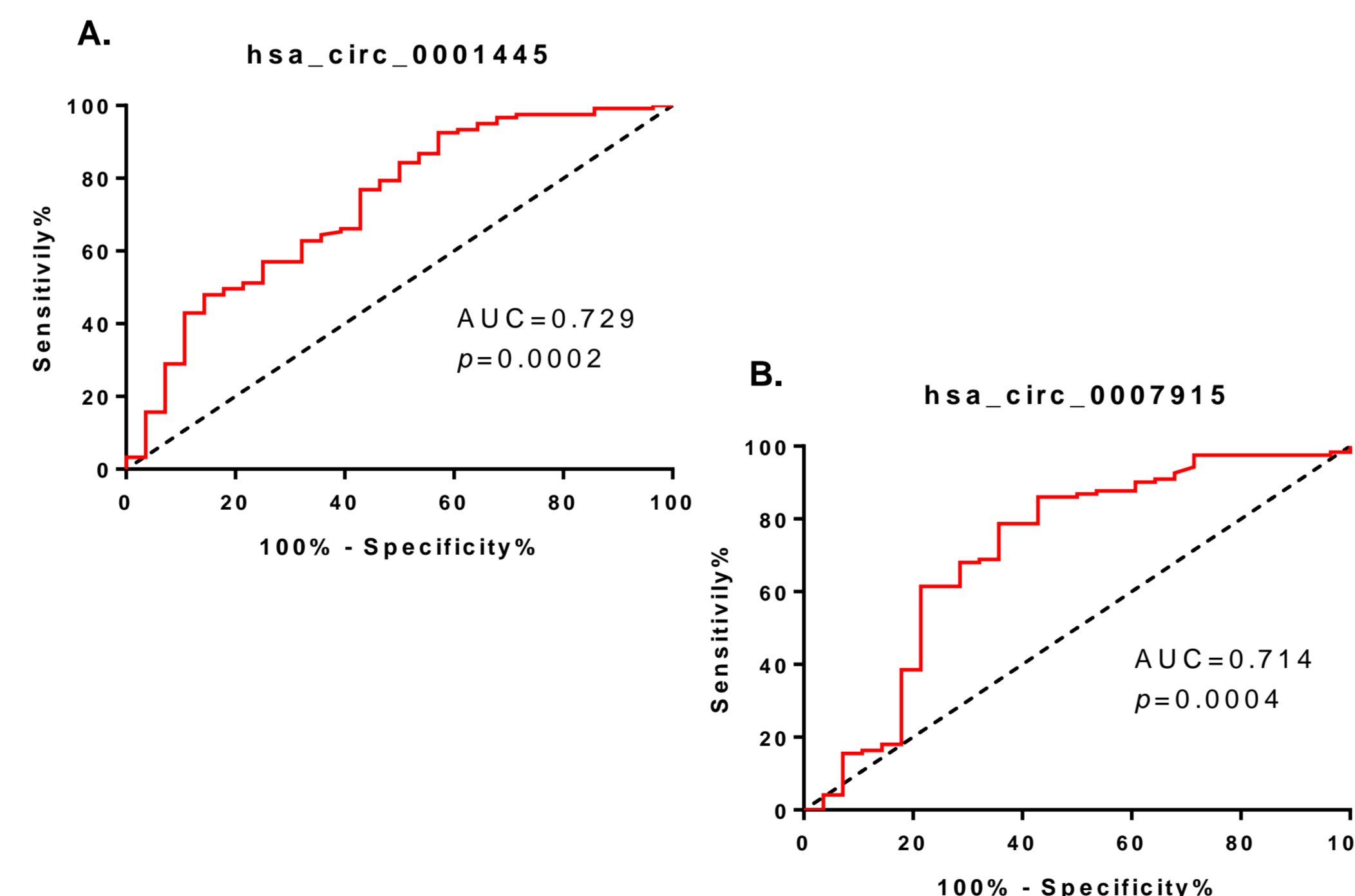
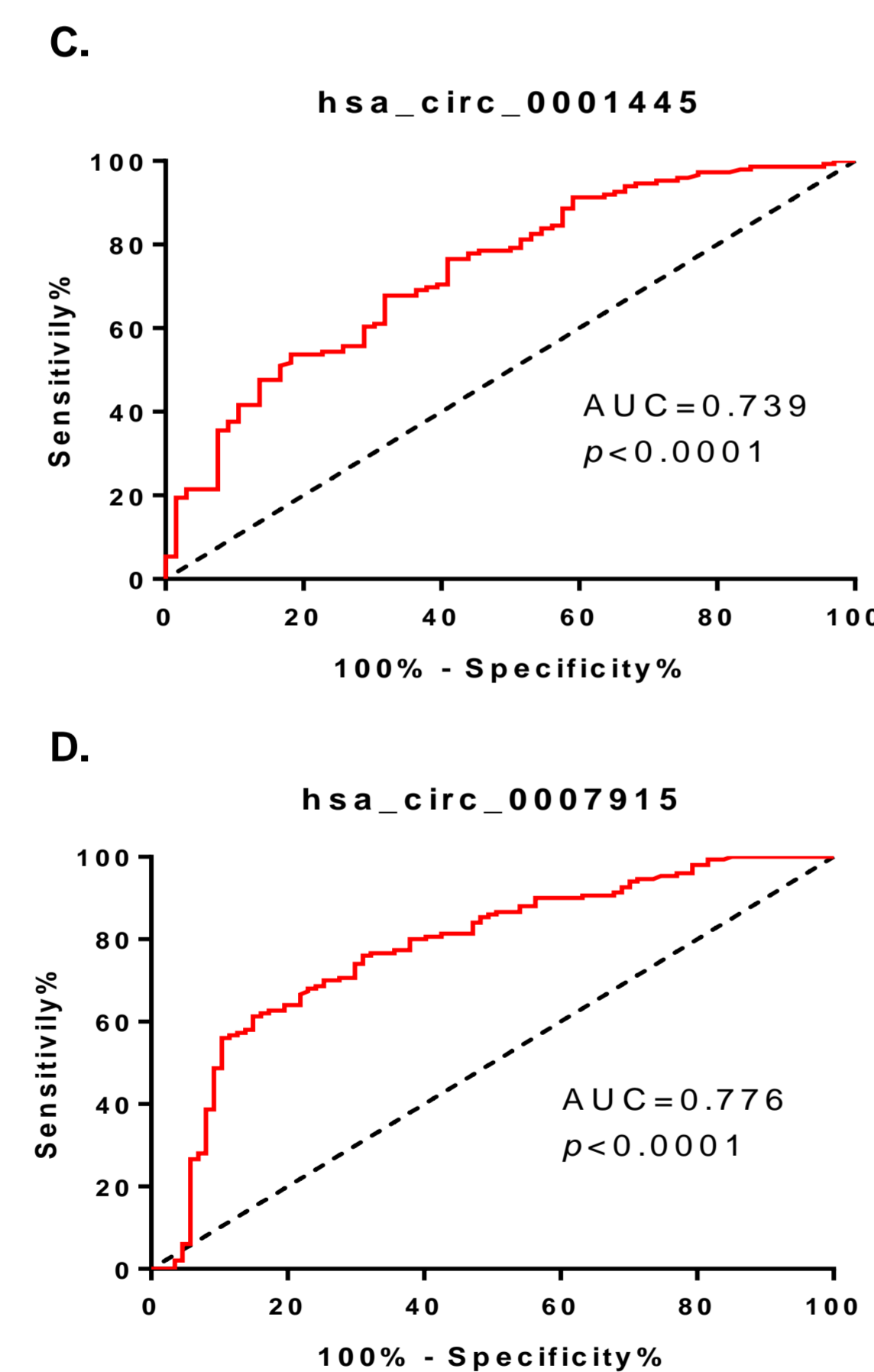


Figure 2. ROC analysis and ROC curves of using hsa_circ_0001445 (A.) and hsa_circ_0007915 (B.) to differentiate patients with CRC in IV stage from patients in stage III.

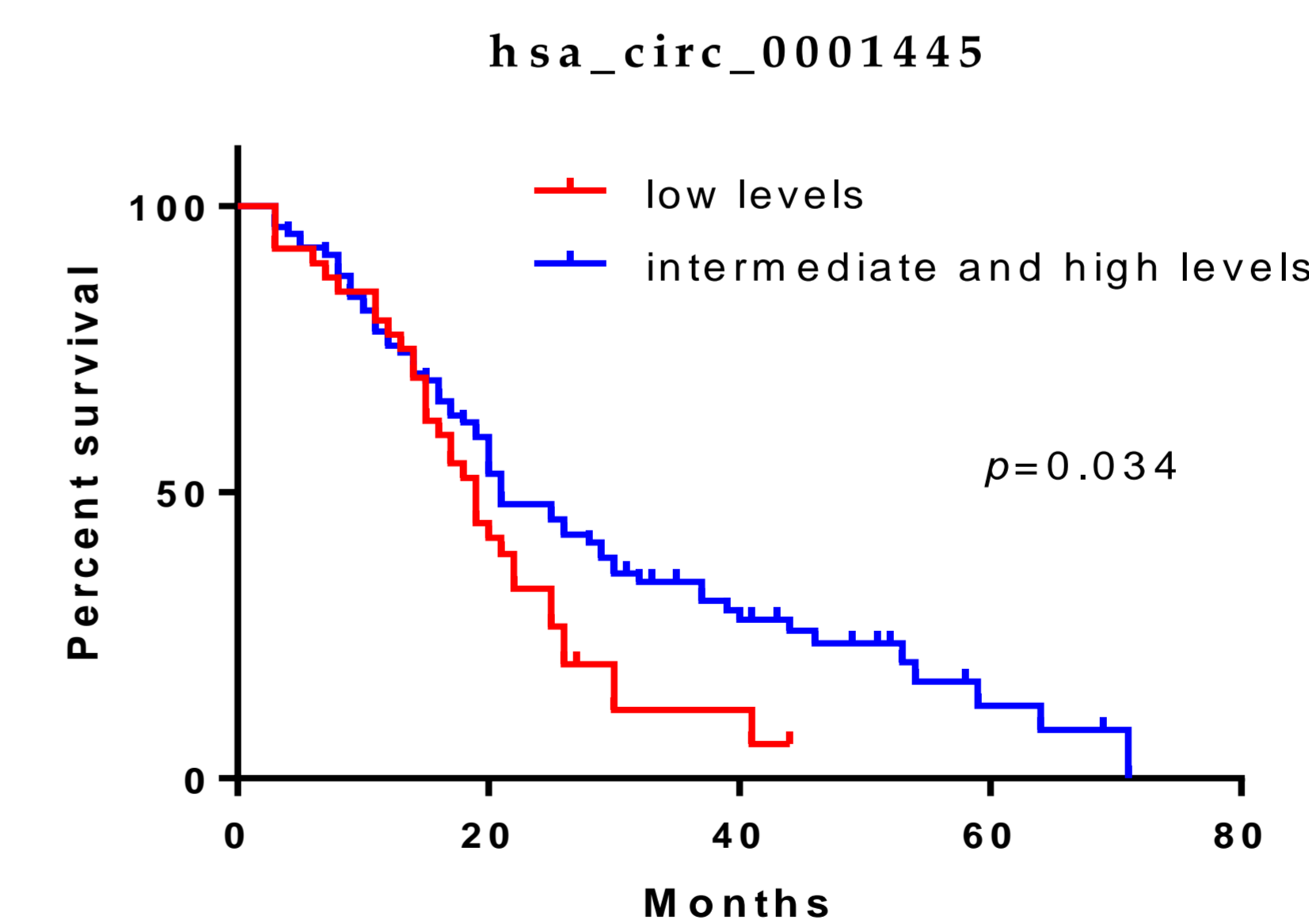


Figure 3. Kaplan-Meier survival analysis for assessment of the hsa_circ_0001445 levels and the overall survival of CRC patients in IV stage.

Table 1. Results of Cox regression analysis for predicting overall survival

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age <65 vs \geq 65	1.12	0.73-1.73	0.59			
Sex male vs female	0.89	0.58-1.37	0.61			
Histological grade low vs high	0.72	0.43-1.19	0.20			
RAS status WT vs M+	0.71	0.46-1.11	0.12			
Liver metastasis no vs yes	0.78	0.43-1.37	0.38			
Peritoneum metastasis no vs yes	0.70	0.40-1.23	0.22			
Lung metastasis no vs yes	0.95	0.59-1.52	0.83			
Primary tumor location left colon vs right colon	0.57	0.36-0.91	0.019	0.61	0.38-0.97	0.036
CEA \leq 2 ULN vs $>$ 2 ULN	0.51	0.32-0.80	0.003	0.52	0.33-0.80	0.004
hsa_circ_0001445 low vs high/intermediate	1.58	1.02-2.45	0.039	1.59	1.02-2.47	0.040

CONCLUSIONS

Plasma expression levels of two new for CRC circRNAs were evaluated. These two circRNAs, hsa_circ_0004515 and hsa_circ_0007915, may be useful to differentiate metastatic from non-metastatic CRC patients. From both only hsa_circ_0001445 has prognostic significance in metastatic patients.

Funding

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