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Original Research Paper

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MCRS1 EXPRESSION MORE IN TUMOR PART AND SERVES AS A POOR PROGNOSTIC FACTOR IN EXTRAHEPATIC CHOLANGIOCARCINOMA

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ABSTRACT

INTRODUCTION: Microporous protein 1 (MCRS1) acts as a cancer gene. MCRS1 is associated with poor prognosis in several types of cancer including colorectal cancer, hepatocellular carcinoma, glioma, and non-small cell lung cancer. In the current study, we are trying to shed light on the role of MCRS1 in the extrahepatic cholangiocarcinoma.

METHODS: We retrospectively selected 13 patients who diagnosed extrahepatic cholangiocarcinoma. All clinical charts and histopathology reports were reviewed for and recoded for age, gender, tumor size, surgical margin status, lymph node metastasis, distant metastasis and TMN staging. All patients were followed for 1~10 years. The median follow-up period was 3.2 years.

RESULTS: The expression level of MCRS1 showed significantly higher in tumor part than non-tumor part. In the Kaplan-Meier survival plot , the high MCRS1 expression group showed poor survival probability with p value of 0.020. The Hazard ratio of MCRS1 showed 8.393 folds in high MCRS1 expression group when compared with low expression group with the borderline p value of 0.05.

CONCLUSION: MCRS1 serves as a poor prognostic factor. Further analysis, no correlation was found in proliferation, apoptosis, angiogenesis and EMT markers. The reason may be the sample size and large-scale study in the future is mandatory.

KEYWORDS : Cholangiocarcinoma, MCRS1, prognosis, immunohistochemical, tissue array

INTRODUCTION

Biliary duct cancer is rare, but it is heterogeneous, and consists of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and gallbladder cancers. They can occur along any part of the bile duct system from the neoplastic proliferation of cholangiocytes. Although the three anatomical groups are classified into different bile duct cancers, it is understood that the patterns of recurrence and prognosis are different [1]. Preclinical trials have strengthened this thinking process, and in the samples of bile duct cancer, the phenotypic characteristics of bile duct cells and progenitor cells were found to be consistent with the anatomical site of origin, confirming heterogeneity between the three groups [2]. Progenitor cells from the canals of Hering have been identified in intrahepatic cholangiocarcinoma, while those within the peribiliary glands have been identified in extrahepatic cholangiocarcinoma, respectively [3-5].

Fragmentary understanding of the molecular features of extrahepatic cholangiocarcinoma has so far been linked to the current state where targeted therapy has not been approved in this cancer type patient [6]. Also, although biliary tract cancer is widely recognized as a separate tumor with different molecular profiles for different anatomical subtypes, there is no data available for subgroup analysis in many biomarker-driven trials, and all bile duct cancers are grouped together. [7] Thus, the lack of stratification by other carcinogenic drivers and the inclusion of patients in the vague definition of biliary tract cancer further complicates the possibility of evaluating the role of targeted therapy in the cohort of biliary tract cancer [8].

Microporous protein 1 (MCRS1, also known as MSP58) is one of these important nucleolar components that contribute to many cellular processes, including transcriptional regulation by interaction with various transcription factors [9-12]. It was identified for the first time as a proliferation-related nucleophile protein p120 interaction partner [13] and it was also shown that MCRS1 acts as a cancer gene in fibroblast transformation assays [14,15]. In the current study, we are trying to shed light on the role of MCRS1 in the extrahepatic cholangiocarcinoma.

METHODS

Patients

We retrospectively selected patients who diagnosed extrahepatic cholangiocarcinoma in Cardinal Tien Hospital between 2003 and 2004. All the patients with extrahepatic cholangiocarcinoma had undergone routine Whipple operation or tumor resection with concurrent regional lymphadenectomy. We excluded patients who had received chemotherapy or radiation therapy before resection. Finally, 13 patients were included in the current study. All clinical charts and histopathology reports were reviewed for and recoded for age, gender, tumor size, surgical margin status, lymph node metastasis, distant metastasis and TMN staging. All patients were followed for 1~10 years. The median follow-up period was 3.2 years. All enrolled patients were de-linked anonymously for protection, and this study protocol was approved by the Institutional Review Board of Cardinal Tien Hospital.

Tissue microarray construction

All tissue samples were routinely fixed in formalin and embedded in paraffin wax at time of diagnosis. All the selected tissue samples were cut for a new slide and undergone standard hematoxylin and eosin (H&E) staining. After choose the tumor and non-tumor parts, punched the regon of interests in paraffin blocks of these samples by using a 2.0-mm punch, and inserted the samples into recipient paraffin blocks to form complete tissue arrays. Sections of 5 m were cut from these complete array blocks and transferred to salinized glass slides.

Histology, immunohistochemistry and scoring

The abovementioned paraffin-embedded tissue array blocks were cut into 5-μm-thick sections for H&E staining.

Immunohistochemicl stain (IHC) staining was performed using a Ventana BenchMark XT automated stainer (Ventana, Tucson, AZ, USA) and primary antibodies for MCRS1 (1:400, rabbit, catalog number: HPA039057, Sigma-Aldrich, Munich, Germany), Ki-67 (1:100, mouse, catalog number: 350503, BioLegend, CA, US), Caspase-3-cleaved (1:100, Rabbit, catalog number: 9664, Cell Signaling, MA, US), CD31 (1:500, rabbit, catalog number: 250590, Abbiotec, CA, US), E-cadherin (1:100, rabbit, catalog number: ab40772, Abcam, MA, US), N-cadherin (1:75, rabbit, catalog number: ab76011, Abcam, MA, US), Fibronectin (1:50, Mouse, catalog number: SC-8422, Santa cruz, TX, US), AKT-phophorylated (1:50, mouse, catalog number: GTX11901, GeneTex, CA, US), ERK-phophorylated (1:200, rabbit, catalog number: AF1018 R&D, MN, US), STAT3-phophorylated (1:50, rabbit, catalog number: ab76315, Abcam, MA, US), and AMPK-phophorylated (1:100, rabbit, catalog number: 2535, Cell signal). All the IHC slides were reviewed, and immunostaining intensity was recorded as 0 for no staining, 1 for faint staining, 2 for moderate staining, and 3 for intense staining. The staining percentage of each core, ranging from 0% to 100%, was also recorded. Then, an H-score ranging from 0 to 300 was calculated by multiplying the staining intensity by the percentage of each core.

Statistical analyses

All statistical analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL). To test for differences between high and low MCRS1 expression, chi-square analysis was performed for categorical variables. To test for the differences of H-score, student t-test was used. Simple bivariant correlation was applied to see the correlation between tumorigenesis markers and MCRS1. Hazard ratio was calculated for the overall survival according to high or low expression of the MCRS1. Finally, Kaplan-Meier survival plot with log-rank test were analyzed. All statistical tests were two-sided, and the results were considered statistically significant when $p < 0.05$.

RESULTS

There were thirteen patients with extrahepatic cholangiocarcinoma included in the current study. The mean ages at diagnosis were 64.8 years and 46.2% of patients was female (Table 1). Elderly, moderate differentiation, T3 /T4 stage, presence of lymph node metastasis and incomplete surgical margin showed more percentage of high expression of MCRS1. Nevertheless, all these findings were not reached statistically significance.

Table 1 Demographic data of the study subjects

		MCRS1			
		Low expression		High expression	
Age (years)	<65	4	(66.7%)	2	(33.3%)
	≧65	2	(28.6%)	5	(71.4%)
Gender	Male	3	(42.9%)	4	(57.1%)
	Female	3	(50.0%)	3	(50.0%)
Differentiation	Well	2	(100.0%)	0	(0.0%)
	Moderate	4	(36.4%)	7	(63.6%)
T stage	T1 / T2	4	(50.0%)	4	(50.0%)
	T3 / T4	2	(40.0%)	3	(60.0%)
Lymph node metastasis	Absence	4	(50.0%)	4	(50.0%)
	Presence	2	(40.0%)	3	(60.0%)
Complete surgical margin	Yes	5	(50.0%)	5	(50.0%)
	No	1	(33.3%)	2	(66.7%)

When MCRS1 correlated with invasiveness markers of proliferation (Ki-67), apoptosis (Caspase 3), angiogenesis (CD31), EMT markers (E-cadherin, N-cadherin, fibronectin), tyrosine kinase of Akt, Erk, STAT3 and AMPK, all were showed non-significant. This may be due to the small sample size in the current study (Table 2).

Table 2 Correlation of MCRS1 and carcinogenesis markers

Biomarkers		r	p
Proliferation	Ki67	-0.256	0.399
Apoptosis	caspase3	0.168	0.584
Angiogenesis	CD31	0.097	0.752
EMT	E-cadherin	-0.113	0.712
	N-cadherin	0.443	0.129
	Fibronectin	0.091	0.767
Tyrosine kinase	pAkt	0.418	0.156
	pErk	0.240	0.429
	pSTAT3	0.316	0.294
	pAMPK	0.052	0.865

EMT, epithelial-mesenchymal transition

Table 3 Overall survival Hazard ratio of the MCRS1

	Hazard ratio (95% CI)		p vale
MCRS1	8.393	(0.999 —70.524)	0.050
Age	2.710	(0.515 —14.259)	0.239
Gender	0.612	(0.117 —3.201)	0.560
Differentiation	50.374	(0.054 —47165.395)	0.262
T stage	0.634	(0.122 —3.283)	0.587
Lymph node metastasis	3.467	(0.676 —17.795)	0.136
Complete surgical margin	2.257	(0.446 —11.427)	0.325

The expression level of MCRS1 showed significantly higher in tumor part than non-tumor part. Other status including late stage than early stage, female than male, and elderly than adult express higher MCRS1 but p value was not reached statistical significance (Figure 1).

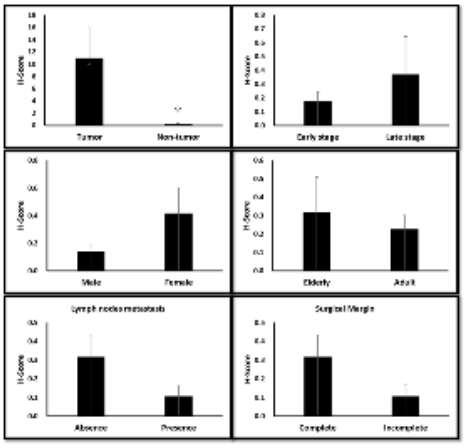

Figure 1 MCRS1 expression levels in different groups

In the Kaplan-Meier survival plot (Figure 2), the high MCRS1 expression group showed poor survival probability with p value of 0.020. The Hazard ratio of MCRS1 showed 8.393 folds in high MCRS1 expression group when compared with low expression group with the borderline p value of 0.05 (Table 3). Finally, representative MCRS1 expression pattern from no staining to strong staining were demonstrated in figure 3.

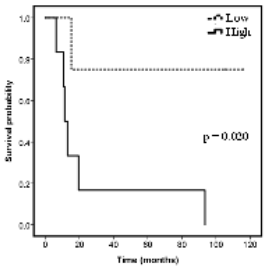

Figure 2 Kaplan-Meier plot of MCRS1 survival probability in extrahepatic cholangiocarcinoma

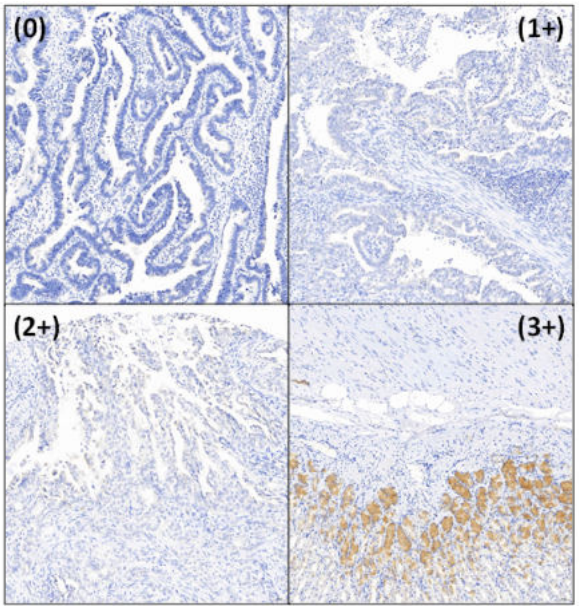

Figure 3 Representative of the MCRS1 expression pattern in extrahepatic cholangiocarcinoma

DISCUSSION

MCRS1 and related protein p78 were first identified as proteins that interact with nucleophile protein p120 and herpes simplex virus 1 infected cell protein 22 (ICP22) [16,17]. MCRS1 is a nucleosome protein that contains a bipartite nuclear localization motif, a nucleosome localization motif coil-coil domain, and a forkhead-related domain. Some other functions of MCRS1 including transforming activity, nucleolar sequestration activity, and telomerase inhibition [18-21]. Proteins with high structural similarity to MCRS1, TOJ3, exhibit transgenic activity, whereas phosphatase and tensin homologues (PTEN) inhibit the transgenic activity of MCRS1 [18,20]. MCRS1 has also been shown to relieve the repressor activity of Daxx, an adapter protein that links Fas signaling to the c-JunNH2 terminal kinase pathway through a nucleolar sequestration mechanism [21]. In addition, the binding and stabilization of MCRS1 has been observed according to the transcription factor STRA13 [22].

MCRS1 links nutrient surplus to the activation of mTORC1 [23]. Its ablation of the intestinal epithelium inhibits the activation of mTORC1, promotes DNA damage and chromosomal abnormalities, inhibits the CDK4/CKD6 axis, reduces the ability to proliferate in the crypt, increases apoptosis and impairs tissue regeneration. Thus, it is suggested that DNA damage activates p53 and releases CDK4/CDK6/pRB/E2F axis activity [24,25]. MCRS1 is associated with poor prognosis in several types of cancer including colorectal cancer [26], hepatocellular carcinoma [27], glioma [28,29], and non-small cell lung cancer [30].

Recurrence of metastatic disseminated disease and drug resistance are the leading causes of poor clinical outcomes in cancer patients, and there is strong evidence that this process is closely related to the epithelium-mesenchymal transition (EMT) [31,32]. One study showed that MCRS1 is a regulator of the EMT program in non-small cell lung cancer cells [33]. Overexpression of MCRS1 induces proliferation of non-small cell lung cancer through the miR-155-Rb1 pathway, so that DNA copy number amplification is one of the mechanisms underlying the overexpression of MCRS1 in non-small cell lung cancer [33]. Another study showed that miR-129-5p can inhibit the survival and invasion of lung cancer cells, which can occur through regulation of the expression of MCRS1, E--cadherin, and vimentin [34].

MCRS1 protein was expressed in the nucleus and cytoplasm of in colorectal carcinoma tubular epithelial cells, and that MCRS1 expression was significantly increased in colon cancer part compared to adjacent non-cancerous tissues [35]. Expression of MCRS1 was positively correlated with depth of invasion, grade, stage, and local recurrence. The survival time was shorter with high MCRS1 expression in colorectal carcinoma patients [36]. This finding was consistent with our findings and we regard MCRS1 as a poor prognostic factor. In conclusion, MCRS1 serves as a poor prognostic factor. Further analysis, no correlation was found in proliferation, apoptosis, angiogenesis and EMT markers. The reason may be the sample size and large-scale study in the future is mandatory.

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