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Original Research Article

Myocardin immunohistochemistry index is associated with clinical prognosis in nasopharyngeal carcinoma: a clinical practice-based cohort study

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Abstract

Purpose: *Recent findings have implicated the role of myocardin re-expression in carcinogenesis. However, the clinical functions of myocardin in nasopharyngeal carcinoma (NPC) is not known yet. The purpose for the cohort research was to investigate whether myocardin re-expression level may predict clinical prognosis in NPC patients.*

Methods: *148 NPC patients were recruited from September, 2005 to September, 2011 with median follow-up time of 4.5 years in a clinical practice setting. At study entry myocardin re-expression of these patients was determined using immunohistochemistry (IHC) and additional 20 normal nasopharyngeal tissues were included as control. Two-sample t-test was used to compare mean myocardin re-expression levels and Chi-square test was used for comparing tumour recurrence rate. Logistic regression analysis was used for tumour local control rate, and log-rank test, Kaplan-Meier estimates and Cox proportional hazard model for disease-free survival and overall survival.*

Results: *Myocardin IHC index was significantly downregulated in NPC samples than in normal nasopharyngeal tissues (mean ± standard deviation, 61.2 ±31.5 vs. 109.9 ±73.6, P= 0.009). However, among NPC patients was observed a roughly V-shaped change of myocardin IHC index according to Tumour T-stage (P=0.067); meanwhile higher IHC level was associated with more tumour recurrence rate in NPC patients (High vs. Low: 21.6% vs. 8.1%; P=0.021). Logistic regression analysis equally showed high myocardin IHC level was an independent risk factor for local tumour control rate regardless of adjustments [High vs. Low: unadjusted Odds Ratio (OR) 0.320, 95% confidence interval (CI): 0.117 to 0.871; P=0.026]. Moreover, higher myocardin IHC level was associated with a marginal but not significant risk increase of disease-free survival [High vs. Low: adjusted Hazard Ratio (HR) 1.760, 95% CI: 0.981 to 3.158; log-rank: P=0.129]. A less obvious trend was observed with regard to overall survival [adjusted HR 1.409, 95% CI: 0.715 to 2.77; log-rank: P=0.745].*

Conclusion: *The study results suggested that high myocardin IHC index level could be a potential clinically prognostic intermediate biomarker for tumour recurrence for NPC patients in routine practice. Large well-designed cohort studies involving IHC re-expression change over time is needed.*

Keywords: *nasopharyngeal carcinoma; prognosis; myocardin; biomarker*

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INTRODUCTION

Myocardin is a key protein in cardiac muscle evolvement and drives transcription through interactions [1, 2]. Recent study suggested that myocardin expressed in other tissues [3] and was in a broad range of pathogenesis of cardiovascular diseases, neuronal degradation symdromes and malignant transformation [3-6]. The molecular mechanism of myocardin involvement in carcinogenesis is complex. Over-expressed myocardin had effects on anti-proliferation specifically targeting G2-M phase arrest and inducing differentiation [7, 8]. Inversely, knockdown of myocardin led to enhanced colony formation and differentiation defect [3]. Recently, myocardin was reported broad interaction with cyclin-dependent kinase inhibitor (CDKI, p21) , NF-κB(p65), p16INK4A (p16), Rb1(Rb) , p53 and TGFβ, which have been well established in cell cycle progression and differentiation [3, 6, 7, 9]. In addition, our previous study suggested myocardin promoter methylation associated with gene silencing is another potential cause of carcinogenesis [10].
Myocardin is found silenced in malignant tumors, sarcomas, epithelial tumors, etc. Milyavsky M et al tested myocardin re-expression in different types of clinical tumour samples searched from Oncomine database and compared to the corresponding normal tissues [6]. The results showed downregulated myocardin in prostate and colon tumour samples. A decrease in myocardin mRNA expression was also seen in sarcoma types [3, 11]. Our previous study initially demonstrated myocardin re-expression in nasopharyngeal carcinoma (NPC) [10].

However, the correlation between myocardin re-expression and prognosis of cancer patients has not been explored up to now. NPC, one of the most common cancers in southern China and South-eastern Asia, is an invasive and rapidly proliferating tumour. Its biomarker for effective diagnosis and prognosis hasn't been verified yet [12]. In current research, we explored myocardin re-expression in patients with NPC as well as firstly evaluated its clinically prognostic value for NPC.

METHODS

Study population and follow-up

Total 148 patients with NPC were enrolled between September, 2005 and September, 2011 at the department of Radiation Oncology, Eye Ear Nose and Throat Hospital of Fudan University, Shanghai, China. The patients who had previous other malignant diagnoses, concurrent malignancies, or secondary tumors, severe infection and systemic autoimmune disease, were excluded. NPC biopsy samples were obtained for Myocardin re-expression measurement. All patients received radiotherapy with or without chemotherapy and/or target therapy thereafter. After study treatment, patients were followed up every 1-2 month for the first year, every 3 months for the second years, and every 6 months thereafter until death, lost to visit or Nov, 2014 (cut-off date for this analysis). If patients were not able to return for visits, telephone call or email follow-up were performed. Follow-up data contained survival status and disease status (disease-free, metastasis or recurrence), along with dates of the events and cause of mortality. Disease-free survival was defined as the time interval from treatment start to the time of the first relapse at any site, death from any cause, or last recorded follow-up visit. Overall survival (OS) was defined as the time interval from treatment start to the time of death from any cause or last recorded follow-up visit.

NPC samples

NPC biopsies were accessed from 148 patients with primary NPC at our department between 2005 and 2011; meanwhile normal nasopharyngeal tissues as control were obtained from 20 non-tumor patients with surgical resections or biopsies.

All samples were histologically verfied with two pathologists in a blind manner. The clinical stage of NPC was decided according to the 7th edition of tumour, node, and metastasis (TNM) classification system of the American Joint Committee on Cancer/Union for International Cancer Control, and histological type was determined in accordance to World Health Organization (WHO) criteria.

Immunohistochemistry assay

Immunohistochemistry stain was performed as described previously [13]. Paraffin-embedded sections of NPC samples were detected with immunohistochemistry stain by a two-step EnVision/HRP technique (Dako Cytomation, Denmark) under the manufacturer's instruction. Polyclonal rabbit antihuman myocardin antibodies (dilution 1:200 for antigen) were obtained from LifeSpan Biosiences, Inc., Seattle, USA (batch number: LS-C153495). The omission of primary antibodies was applied as the negative control. For myocardin stain, cytoplasm stained with brown was signed as positive. The re-expression of myocardin was quantitatively measuring by an Olympus CX31 biomicroscope

with computer-aided images analysis system (Qiu Wei Inc, Shanghai, China). The digital images were accessed with CANON A640 camera. The positive area and optical density (OD) of myocardin-positive cells were decided through calculating three randomly selected microscopic fields (25 ×10) for each slide. The IHC index was described as average integral optical density (AIOD) (AIOD =positive area × OD/total area).

Statistical analysis

All statistical analyses were processed with R 3.1.2 [14]. Continuous variables were displayed with summary statistics including mean and standard deviation (SD), while categorical variables were shown with frequency (%). Two-sample t-test or analysis of variance for continuous data and Pearson's chi-square test for categorical data were performed for comparisons. The non-parametric Kaplan-Meier (KM) estimates and log-rank test were performed for survival data analysis. Cox proportional Hazard Model was applied to the survival data without any adjustment or stratified by Clinical Stage and also adjusted for age, gender (Female vs. Male), T stage (T1-T2 vs. T3-T4), N stage (N0 vs. N1-N3), chemotherapy (Yes vs. No), radiotherapy (Conformal Radiation Therapy, CRT vs. Intensity Modulated Radiation Therapy, IMRT), target Treatment (Yes vs. No), prior radiotherapy (Yes vs. No). Logistic regression analysis without adjustment or with different combinations of adjustment factors, which included myocardin IHC index, age, gender, T stage, N stage, clinical stage and various treatments as risk factors, was applied to evaluate whether myocardin IHC level is an independent risk factor for NPC recurrence. P value less than 0.05 was identified as statistically significant different.

RESULTS

Demographic data and follow-up status

Total 148 patients with NPC were enrolled, among which 12 patients (8.1%) received radiotherapy prior to the study. Among them, the mean age was 48.5±12.7 years, male was 110 (77.0%) and patients with clinical stage I-IV were 11 (7.4%), 33 (22.3%), 72(48.6%), 32 (21.6%) respectively. By the end of this study analysis, the median follow-up time (Q1, Q3) was 4.5 (3.1, 7.1) years, patients lost to follow-up 23 (15.5%), death 36 (24.3%) and still alive 89 (60.1%).

Myocardin IHC index in human issues

Myocardin re-expression was evaluated using IHC index. The myocardin IHC index was obviously lower in NPC patients compared with that in non-tumor patients (61.2 ±31.5 vs.109.9 ±73.6; P=0.009). This data indicated that myocardin re-expression was downregulated in NPC patients.

Further analysis of these NPC patients showed that the myocardin re-expression IHC index was seemingly associated with tumour T stage (P=0.0667, Table 1). In parallel with this finding, the mean myocardin IHC index in NPC patients without recurrence seems lower than that in patients with recurrence (58.5 ± 23.9 vs. 76.8 ± 57.2). And a less obvious trend in myocardin IHC index was observed according to disease-free status (disease free vs. non disease free: 58.4 ±22.1 vs. 66.7 ± 44.3) or prior radiotherapy (No vs. Yes: 60.9 ±30.8 vs. 65.0 ± 40.6).

Clinicopathological characteristics according to myocardin IHC level

Table 2 summarizes the clinicopathological parameters in 148 NPC patients according to myocardin IHC level (Low (≤ IHC Median) vs. High (> IHC Median)). It was found that high myocardin IHC level was associated with higher tumor recurrence in NPC patients (21.6% vs. 8.1%; P=0.021). Age, gender, N-stage, clinical stage, or tumor metastasis status were not significantly associated with myocardin IHC levels.

Table 1: Myocardin Immunohistochemisty Index According to Tumour T-staging in NPC patients

	Tumour T-Staging					
	T1 (N=24)	**T2 (N=53)**	**T3 (N=39)**	**T4 (N=32)**	**Overall**	**P-value**
IHC index					0.0667	
Mean (SD)	74.2 (42.6)	53.9 (16.3)	61.0 (32.0)	64.0 (37.9)		
Median	63.2	53.3	60.2	59.7		
Q1 : Q3	54.4 : 78.2	46.7 : 63.6	41.4 : 74.2	36.8 : 75.6		
Min : Max	45.2 : 260.8	7.6 : 98.9	3.4 : 165.8	16.7 : 178.7		

Abbreviations: Immunohistochemisty, IHC; Nasopharyngeal Carcinoma, NPC

Table 2: Clinicopathological Parameters in NPC Patients According to Myocardin IHC Level

	All (N=148)	Myocardin IHC Level		P-value
		Low (≤Median) (N=74)	High(> Median) (N=74)	
Age at Treatment Start (years)				
Mean (SD)	48.5 (12.7)	48.6 (13.2)	48.5 (12.3)	0.9385
Median	50.0	51.0	49.0	
Q1 : Q3	40.0 : 57.0	39.0 : 57.0	41.0 : 57.0	
Min : Max	13 : 80	13 : 76	14 : 80	
Age group (years) [n (%)]				
<65	136 (91.9%)	67 (90.5%)	69 (93.2%)	0.5470
≥65	12 (8.1%)	7 (9.5%)	5 (6.8%)	
Gender [n (%)]				
Female	34 (23.0%)	18 (24.3%)	16 (21.6%)	0.6959
Male	114 (77.0%)	56 (75.7%)	58 (78.4%)	
N Stage [n(%)][b]				
N0	31 (20.9%)	12 (16.2%)	19 (25.7%)	0.3999
N1	50 (33.8%)	26 (35.1%)	24 (32.4%)	
N2	65 (43.9%)	35 (47.3%)	30 (40.5%)	
N2b	1 (0.7%)	0	1 (1.4%)	
N3	1 (0.7%)	1 (1.4%)	0	
Clinical Stage [n (%)]				
I	11 (7.4%)	4 (5.4%)	7 (9.5%)	0.5456
II	33 (22.3%)	19 (25.7%)	14 (18.9%)	
III	72 (48.6%)	37 (50.0%)	35 (47.3%)	
IV	32 (21.6%)	14 (18.9%)	18 (24.3%)	
N Stage [n (%)]				
N0	31 (20.9%)	12 (16.2%)	19 (25.7%)	0.1574
N1-N3	117 (79.1%)	62 (83.8%)	55 (74.3%)	
Clinical Stage [n (%)]				
I-II	44 (29.7%)	23 (31.1%)	21 (28.4%)	0.7191
III-IV	104 (70.3%)	51 (68.9%)	53 (71.6%)	
Recurrence [n (%)]				
Yes	22 (14.9%)	6 (8.1%)	16 (21.6%)	**0.0209**
No	126 (85.1%)	68 (91.9%)	58 (78.4%)	
Metastasis [n (%)]				
Yes	16 (10.8%)	8 (10.8%)	8 (10.8%)	1.0000
No	132 (89.2%)	66 (89.2%)	66 (89.2%)	

Note: Myocardin IHC index has a median of 58.5. Abbreviations: Immunohistochemisty, IHC; Nasopharyngeal Carcinoma, NPC

Study treatment

Table 3 summarizes study treatments according to myocardin IHC level. It seems that no any significant associations were noted between IHC level and these study treatments.

The region of the clear microemulsions in the pseudo-ternary phase diagrams appeared to contain volume fractions of water and IPM less of than 0.5, but higher than 0.5 of Tween 80. On the other hand, the addition of atorvastatin had no significant effect on the pseudo-ternary phase diagrams.

The rheologic properties of atorvastatin-loaded microemulsions comply with Newtonian fluids' characteristics. All the MEs had droplets' sizes of less than 100 nm within the limit of colloidal dispersions, and that, apart from the Newtonian fluid property, can facilitate the absorption of atorvastatin through the skin.

Association of myocardin IHC level with clinical prognosis

Logistic regression analysis showed that high myocardin IHC level was an independent risk factor for local control rate (High vs. Low: unadjusted OR 0.320, 95% confidence interval (CI): 0.117 to 0.871, P=0.026, Table 4). Furthermore, consistent resulting trend was noted with all P values <0.05 even adjusting different combination of adjustment factors (Table 4) or adjusting for age, gender, T-stage, N-stage, clinical stage and treatment types as well (Table 5). In addition, the results also showed prior radiotherapy (adjusted OR 0.044, 95% CI: 0.008 to 0.242, P=0.000) predict more NPC recurrence during the current study observation period (Table 5).

Table 3: Study Treatments in NPC Patients According to Myocardin IHC Level

	All(N=148)	Myocardin IHC Level		P-value
		Low (≤Median) (N=74)	High (> Median) (N=74)	
Treatment Duration (months)				
Mean (SD)	1.6 (0.5)	1.6 (0.5)	1.6 (0.6)	0.3959
Median	2.0	2.0	2.0	
Q1 : Q3	1.0 : 2.0	1.0 : 2.0	1.0 : 2.0	
Min : Max	1 : 3	1 : 3	1 : 3	
Chemotherapy [n(%)]				
No	14 (9.5%)	6 (8.1%)	8 (10.8%)	0.5743
Yes	134 (90.5%)	68 (91.9%)	66 (89.2%)	
Radiotherapy Type [n(%)]				
CRT	124 (83.8%)	63 (85.1%)	61 (82.4%)	0.6556
IMRT	24 (16.2%)	11 (14.9%)	13 (17.6%)	
Target Therapy [n(%)]				
No	81 (54.7%)	36 (48.6%)	45 (60.8%)	0.1372
Yes	67 (45.3%)	38 (51.4%)	29 (39.2%)	
Radiotherapy Only [n(%)]				
No	134 (90.5%)	68 (91.9%)	66 (89.2%)	0.5743
Yes	14 (9.5%)	6 (8.1%)	8 (10.8%)	
Radiotherapy plus Chemotherapy				
No	14 (9.5%)	6 (8.1%)	8 (10.8%)	0.5743
Yes	134 (90.5%)	68 (91.9%)	66 (89.2%)	
Radiotherapy plus Chemotherapy and Target Therapy [n (%)]				
No	81 (54.7%)	36 (48.6%)	45 (60.8%)	0.1372
Yes	67 (45.3%)	38 (51.4%)	29 (39.2%)	

Note: Myocardin IHC index has a median of 58.5. Abbreviations: Conformal Radiation Therapy, CRT; Immunohistochemisty, IHC; Intensity Modulated Radiation Therapy, IMRT; Nasopharyngeal Carcinoma, NPC

Table 4: Logistic Regression of Myocardin IHC Level (> Median vs. ≤Median) in NPC Patients Related to Local Control Rate According to Different Adjustments

Adjusted for	Odds Ratio	95% CI	P-value
No Adjustment	0.320	0.117, 0.871	**0.0257**
Clinical Stage (I-II vs. III-IV)	0.320	0.117, 0.880	**0.0273**
Clinical Stage (I-II vs. III-IV), Target Therapy (Yes vs. No)	0.285	0.102, 0.803	**0.0175**
Clinical Stage (I-II vs. III-IV), Radiotherapy Type (CRT vs. IMRT)	0.317	0.115, 0.871	**0.0260**
Clinical Stage (I-II vs. III-IV), Target Therapy (Yes vs. No), Radiotherapy Type (CRT vs. IMRT)	0.264	0.092, 0.758	**0.0134**
Clinical Stage (I-II vs. III-IV), Target Therapy (Yes vs. No), Radiotherapy Type (CRT vs. IMRT), Prior Radiotherapy (Yes vs. No)	0.198	0.057, 0.687	**0.0107**

Note: Myocardin IHC Level as categorical data. Abbreviations: Confidence Interval, CI; Conformal Radiation Therapy, CRT; Immunohistochemisty, IHC; Intensity Modulated Radiation Therapy, IMRT; Nasopharyngeal Carcinoma, NPC

KM curve revealed that NPC patients with high myocardin IHC level achieved a poor 5-year disease free survival rate (High vs. Low: 61.7%, 95% CI: 50.1 to 73.2%; 72.9%, 95% CI: 62.4 to 83.4%, respectively, Figure 1). Similarly, high myocardin IHC level was associated with a marginal but not significant risk increase of disease-free survival (unadjusted Hazard Ratio (HR) 1.54.

5, 95% CI: 0.877 to 2.720 or adjusted HR 1.760, 95% CI: 0.981 to 3.158; log-rank P=0.129, Figure 1). A less obvious trend was observed when with regard to overall survival (High vs. Low: adjusted HR 1.409, 95% CI: 0.715 to 2.77; log-rank P=0.745, Figure 2).

Table 5: Logistic Regression of Risk Factors Related to NPC recurrence rate (Full Model)

Factors		Odds Ratio	95% CI	P-value
Intercept				0.3358
Age (years)	Per one year increase	1.007	0.966, 1.050	0.7289
Gender	Male vs. Female	0.765	0.151, 3.876	0.7466
T Stage	T1-T2 vs. T3-T4	0.922	0.262, 3.247	0.8992
N Stage	N0 vs. N1-N3	1.791	0.382, 8.398	0.4599
Clinical Stage	I-II vs. III-IV	5.181	0.652, 41.149	0.1197
Chemotherapy	Yes vs. No	1.314	0.107, 16.209	0.8313
Radiotherapy Type	CRT vs. IMRT	0.763	0.160, 3.639	0.7343
Target Therapy	Yes vs. No	0.875	0.240, 3.193	0.8393
Prior Radiotherapy	Yes vs. No	0.044	0.008, 0.242	0.0003
Myocardin IHC Level	> Median vs. ≤Median	0.185	0.052, 0.661	0.0094

Note: Myocardin IHC Level as categorical data. Abbreviations: Confidence Interval, CI; Conformal Radiation Therapy, CRT; Immunohistochemisty, IHC; Intensity Modulated Radiation Therapy, IMRT

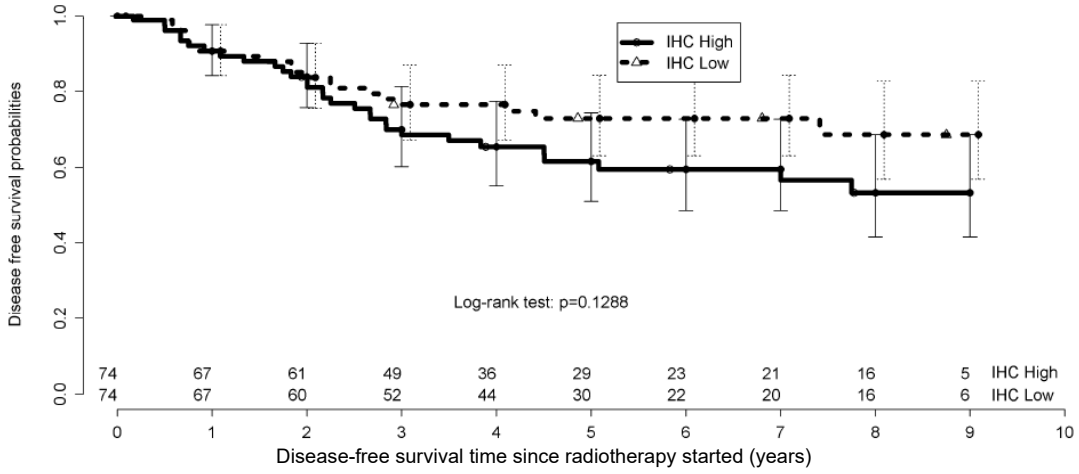

Figure 1 Kaplan-Meier Curves for Disease-free Survival According to Myocardin IHC Level [IHC High, >Median (58.5) vs. IHC Low, ≤ Median] in 148 NPC Patients. Abbreviations: Immunohistochemisty, IHC

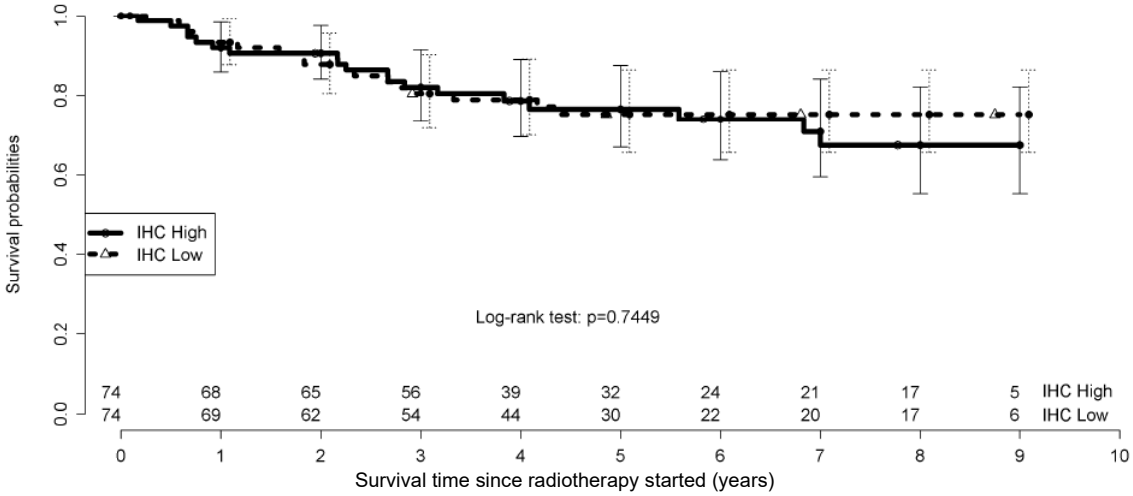

Figure 2 Kaplan-Meier Curves for Overall Survival According to Myocardin IHC Level [IHC High, >Median (58.5) vs. IHC Low, ≤ Median] in 148 NPC Patients. Abbreviations: Immunohistochemisty, IHC

DISCUSSION

Plenty of reports have revealed that myocardin plays a vital role in cell proliferation and differentiation, indicating its involvement in carcinogenesis. In accordance with this, myocardin re-expression was frequently found repressed in a variety of human cancer samples and malignant cells [6]. Our previous study also showed myocardin mRNA re-expression is inactivated in NPC cell lines and primary NPC biopsies [10]. However, the association of myocardin re-expression with prognosis remains unknown currently. In our research, myocardin re-expression was detected through immunohistochemistry and its association with prognosis of human NPC was examined. The data reconfirmed lower myocardin re-expression in NPC biopsies when compared to normal nasopharyngeal tissue. Notable association was also found between myocardin re-expression as well as NPC recurrence or local tumour control rate, independent of other clinical parameters.

Myocardin is an important cell cycle and differentiation regulator by interfering with multiple factors including (CDKI, p21), NF-κB(p65), p16INK4A (p16), Rb1(Rb), p53 and TGFβ, et al [3, 6, 7, 9]. The occurrence of NPC was relevant to inactivation of cell cycle-dependent kinase inhibitors, including p16 and p27 and overexpression of NF-κB, CDK3 and CDK4, indicating that abnormal re-expression of cell cycle-associated proteins and protein kinases took part in NPC cell proliferation and malignant transformation [15-22]. In our previous study as well as the present study, the myocardin re-expression was downregulated in NPC samples versus normal nasopharyngeal tissue, indicating myocardin may be involved in NPC pathogenesis and has the potential to be a biomarker for early diagnosis.

Clinically, we found that NPC patients with non-recurrence or disease free had lower myocardin re-expression than those patients with recurrence or non-disease free. More obviously, it was demonstrated that high myocardin re-expression level was an independent risk factor for tumour local control rate and also increased risk of disease-free survival and even overall survival. Our previous report [10] showed that myocardin re-expression would reactivate in NPC cells by the demethylating agent 5-aza-2'-deoxycytidine (5-aza-dC) administration. Given these, on the one hand, it is possible that patients with tumour recurrence more tend to use some demethylating agents before this study, which may therefore result into myocardin re-expression to some extent. Because of the lack of complete prior drug treatment information before study treatments or lack of re-testing results of myocardin during the study, we did not explore any relationships of drug- myocardin re-expression in NPC patients. However, it was noted in the study that prior radiotherapy did not contribute myocardin IHC index at study entry but increased risk of NPC recurrence rate. Notwithstanding this, we still observed from this study that patients with higher IHC level tend to recur tumour (P=0.009) regardless of prior radiotherapy or subsequent treatments and so on. On the other hand, these patients with tumour recurrence will often experience poor clinical prognosis. Taken together, there should be some intrinsic or extrinsic mechanisms which could facilitate myocardin re-expression and then myocardin IHC level in NPC patients when in parallel with natural tumour progression. This is probably the biological reasons behind a roughly V-shaped change we observed in myocardin IHC index according to tumour T stage. As a consequence, high myocardin IHC level due possibly to long-time treatment or progressive disease is relevant to poor prognosis in patients when tumour inhibition effect due to myocardin re-expression is not dominant during tumour progression. Hence, we proposed that myocard re-expression in NPC likely induced by 5-aza-dC could still have an inhibition effect [7, 8], which possibly behaves like Kank1- a potential NPC inhibitor [23]. In addition, myocardin is different from insulin receptor substrate 1 which is highly expressed in NPC than in non-cancerous nasopharyngeal control tissues although both of them could be used as an independent biomarker for associating unsatisfied prognosis of NPC [24].

For the time being, the prognosis of NPC is unsatisfied. Discovery of biomarkers for predicting metastasis, recurrence and survival time of NPC is of great importance for improving patients' prognosis [12]. The present data implicated clinical significance of myocardin re-expression as potential diagnostic and prognostic intermediate biomarker for NPC patients; however, several limitations should be taken into account. Firstly, myocardin re-expression showed significant clinical prognosis effects on tumour recurrence rate or local control rate. Nevertheless, clinical effects on overall survival or disease free survival were not demonstrated clearly. This may probably attribute to the small sample size of the experiment. Secondly, 5-year overall survival is an important clinical outcome for NPC evaluation. However, 4.5 years of median follow-up time in the present study seems a bit shorter to observe sufficient clinical events of interest even though the longest time is

up to 9.1 years for several study patients. Lastly, NPC pathogenesis is complex and involves multiple factors during the carcinogenesis, metastasis and recurrence. Hence myocardin downregulation alone is not sufficient for the NPC formation and re-expression for disease prognosis either. Identification of such cooperative pathways will be our subsequent research directions, especially those intertwined mechanisms behind myocardin downregulation and re-expression. As a possible trigger of myocardin re-expression, the prior treatment information was, unfortunately, unable to be systematically collected in the present study since patients in China may visit different hospitals freely and accordingly have separate medical records.

CONCLUSION

The results of this study clearly showed downregulation of myocardin re-expression in NPC patients. Our study is the first research to reveal the association of myocardin re-expression with NPC prognosis, displaying that high myocardin re-expression is an independent predictor for tumour recurrence. Our findings implicated myocardin may be a potential prognostic biomarker for NPC in routine clinical practice. We designed large-scale cohort studies involving repeated measurement of IHC index over time for treatment naïve patients are warranted in the future. It would be valuable to investigate how routine drug treatments affect myocardin re-expression among NPC patients.

Abbreviations

NPC: nasopharyngeal carcinoma; IHC: immunohistochemistry; OS: overall survival; WHO: World Health Organization; AIODA: average integral optical density; SD: standard deviation; CRT: conformal radiation therapy; IMRT: intensity modulated radiation therapy

Ethics approval and consent to participate

Our study group has strictly abided by the principles of Helsinki Declaration. The NPC bio-banking program was approved by Eye Ear Nose and Throat Hospital of Fudan University. Written informed consent was obtained from all participants. We made sure that all of the participants were voluntary, and their medical documents to be saved securely. Permission to use samples from these participants as well as further follow-up has been granted by the Ethics Review Committee of Eye Ear Nose and Throat Hospital of Fudan University (No. KJ2011-07) . Any published reports involved in the study would not reveal the participant' identification.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Fu Chen designed, carried out the literature search, and analysed the work, Zhe Zhang conceptualised the work, carried out the data acquisition and manuscript review, Xin-Mao Song did the manuscript editing and statistical analysis, Xue Xiao -define the intellectual content, Xiao-Ying Zhou was also involved in literature search while Sheng-Zi Wang prepared the manuscript. All authors read and approved the paper for publication.

REFERENCES

1. *Pipes GC., Creemers EE, Olson EN. The myocardin family of transcriptional coactivators: versatile regulators of cell growth, migration, and myogenesis, Genes Dev, 20 (2006) 1545-1556.*
2. *Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, Krieg PA, Olson EN. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor, Cell, 105 (2001) 851-862.*
3. *Milyavsky Shats MI, A. Cholostoy, R. Brosh, Y. Buganim, L. Weisz, I. Kogan, M. Cohen, M. Shatz, S. Madar, E. Kalo, N. Goldfinger, J. Yuan, S. Ron, K. MacKenzie, A. Eden, V. Rotter, Inactivation of myocardin and p16 during malignant transformation contributes to a differentiation defect, Cancer Cell, 11 (2007) 133-146.*
4. *Li HJ, Z. Haque, Q. Lu, L. Li, R. Karas, M. Mendelsohn,*

Steroid receptor coactivator 3 is a coactivator for myocardin, the regulator of smooth muscle transcription and differentiation, Proc Natl Acad Sci U S A, 104 (2007) 4065-4070.

5. *Chow N, R.D. Bell, R. Deane, J.W. Streb, J. Chen, A. Brooks, W. Van Nostrand, J.M. Miano, B.V. Zlokovic, Serum response factor and myocardin mediate arterial hypercontractility and cerebral blood flow dysregulation in Alzheimer's phenotype, Proc Natl Acad Sci U S A, 104 (2007) 823-828.*
6. *Shats I, M. Milyavsky, A. Cholostoy, R. Brosh, V. Rotter, Myocardin in tumor suppression and myofibroblast differentiation, Cell Cycle, 6 (2007) 1141-1146.*
7. *Tang RH, X.L. Zheng, T.E. Callis, W.E. Stansfield, J. He, A.S. Baldwin, D.Z. Wang, C.H. Selzman, Myocardin inhibits cellular proliferation by inhibiting NF-kappaB(p65)-dependent cell cycle progression, Proc Natl Acad Sci U S A, 105 (2008) 3362-3367.*
8. *Chen J, C.M. Kitchen, J.W. Streb, J.M. Miano, Myocardin: a component of a molecular switch for smooth muscle differentiation, J Mol Cell Cardiol, 34 (2002) 1345-1356.*
9. *Kimura Y, T. Morita, K. Hayashi, T. Miki, K. Sobue, Myocardin functions as an effective inducer of growth arrest and differentiation in human uterine leiomyosarcoma cells, Cancer Res, 70 (2010) 501-511.*
10. *Chen F, Y. Mo, H. Ding, X. Xiao, S.Y. Wang, G. Huang, Z. Zhang, S.Z. Wang, Frequent epigenetic inactivation of Myocardin in human nasopharyngeal carcinoma, Head Neck, 33 (2011) 54-59.*
11. *[Rhodes DR, J. Yu, K. Shanker, N. Deshpande, R. Varambally, D. Ghosh, T. Barrette, A. Pandey, A.M. Chinnaiyan, Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression, Proc Natl Acad Sci U S A, 101 (2004) 9309-9314.*
12. *Xiao L, T. Xiao, Z.M. Wang, W.C. Cho, Z.Q. Xiao, Biomarker discovery of nasopharyngeal carcinoma by proteomics, Expert Rev Proteomics, 11 (2014) 215-225.*
13. *Gu K, J.D. Zhao, Z.G. Ren, N.Y. Ma, S.T. Lai, J. Wang, J. Liu, G.L. Jiang, A natural process of cirrhosis resolution and deceleration of liver regeneration after thioacetamide withdrawal in a rat model, Mol Biol Rep, 38 (2011) 1687-1696.*
14. *Team R, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing:Vienna, Austria., (2014).*
15. *[Hofmann F, Livingston DM. Differential effects of cdk2 and cdk3 on the control of pRb and E2F function during G1 exit, Genes Dev, 10 (1996) 851-861.*
16. *Baba Y, M. Tsukuda, I. Mochimatsu, S. Furukawa, H. Kagata, K. Satake, S. Koshika, Y. Nakatani, M. Hara, Y. Kato, Y. Nagashima, Reduced expression of p16 and p27 proteins in nasopharyngeal carcinoma, Cancer Detect Prev, 25 (2001) 414-419.*
17. *Pan Y, Q. Zhang, L. Tian, X. Wang, X. Fan, H. Zhang, F.X. Claret, H. Yang, Jab1/CSN5 negatively regulates p27 and plays a role in the pathogenesis of nasopharyngeal carcinoma, Cancer Res, 72 (2012) 1890-1900.*
18. *Shih LC, C.W. Tsai, M.H. Tsai, Y.A. Tsou, W.S. Chang, F.J. Li, M.H. Lee, D.T. Bau, Association of cyclin D1 genotypes with nasopharyngeal carcinoma risk, Anticancer Res, 32 (2012) 1093-1098.*
19. *Acikalin MF, D. Etiz, M.K. Gurbuz, E. Ozudogru, F. Canaz, E. Colak, Prognostic significance of galectin-3 and cyclin D1 expression in undifferentiated nasopharyngeal carcinoma, Med Oncol, 29 (2012) 742-749.*
20. *Wang L, H.Y. Hu, Y.L. Lin, Z.X. Zhao, L. Tan, P. Yu, H.J. Wan, Z. Jin, D. Zheng, CDK3 expression and its clinical significance in human nasopharyngeal carcinoma, Mol Med Rep, 9 (2014) 2582-2586.*
21. *Zhuang M, M. Zhao, H. Qiu, D. Shi, J. Wang, Y. Tian, L. Lin, W. Deng, Effusanin E suppresses nasopharyngeal carcinoma cell growth by inhibiting NF-kappaB and COX-2 signaling, PLoS One, 9 (2014) e109951.*
22. *Wang L, R. Ma, Z. Kang, Y. Zhang, H. Ding, W. Guo, Q. Gao, M. Xu, Effect of IL-17A on the migration and invasion of NPC cells and related mechanisms, PLoS One, 9 (2014) e108060.*
23. *Luo FY, S. Xiao, Z.H. Liu, P.F. Zhang, Z.Q. Xiao, C.E. Tang, Kank1 reexpression induced by 5-Aza-2'-deoxycytidine suppresses nasopharyngeal carcinoma cell proliferation and promotes apoptosis, Int J Clin Exp Pathol, 8 (2015) 1658-1665.*
24. *Luo J, Q. Wen, J. Li, L. Xu, S. Chu, W. Wang, L. Shi, G. Xie, D. Huang, S. Fan, Increased expression of IRS-1 is associated with lymph node metastasis in nasopharyngeal carcinoma, Int J Clin Exp Pathol, 7 (2014) 6117-6124..*