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ORIGINAL ARTICLE

Evaluation of gene expression level of CDC5L and MACC1 in poor prognosis and progression of osteosarcoma

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Abstract Current evidences have indicated that osteosarcoma is strongly associated with abnormal genetic and epigenetic changes that lead to the abnormal expression of oncogenes or methylation of tumor suppressor genes. In the present study, MACC1 and CDC5L mRNA levels in the patients with osteosarcoma were evaluated using quantitative real-time PCR. Our results demonstrated that CDC5L mRNA levels were higher in tumor tissues than in adjacent normal tissues (2.713 ± 0.738 vs. 1.071 ± 0.629; $P < 0.05$). Moreover, MACC1 was upregulated in tumor bone tissues than in adjacent normal tissues (3.221 ± 0.624 vs. 1.427 ± 0.456; $P < 0.05$). Our result demonstrated that high expression of CDC5L was significantly related to advanced TNM stage ($P = 0.032$). No significant difference was determined between CDC5L mRNA expression and other clinicopathological parameters including age, gender, tumor diameter, location, tumor grade, and histological type. In addition, overexpression of MACC1 was strongly correlated with advanced TNM stage ($P = 0.027$) and high tumor grade ($P = 0.035$). Our findings indicated that mRNA level of CDC5L is correlated with advanced TNM stage, and MACC1 may be involved in progression of osteosarcoma.

Keywords Osteosarcoma · Expression · PCR · CDC5L · MACC1

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Introduction

Osteosarcoma is a primary malignancy among children and young adults that is linked to risk of metastasis in patients [1, 2]. Despite treatments and therapeutic strategies including chemotherapy and surgery, the 5-year survival rate of primary osteosarcoma was estimated to be only 50–60 % [3, 4]. It has been previously indicated that the incidence of osteosarcoma is correlated with abnormal expression of genes [5].

Metastasis-associated in colon cancer (MACC1) is located on human chromosome 7 (7p21.1) that is defined as an independent prognostic predictor of metastasis and metastasis-free survival [4].

Overexpression of MACC1 has been found in many types of tumors, including gastric carcinoma, breast carcinoma, ovarian cancer, and hepatocellular carcinoma [6–8]. Furthermore, higher expression of MACC1 was reported to be markedly associated with poor prognosis and metastasis in many kinds of malignancies, such as gastric carcinoma, esophageal cancer, lung cancer, and colon cancer [9–11].

Cell division cycle 5-like (Cdc5L) is an important component of the spliceosome complex and plays an important role in the DNA damage response following exposure to genotoxic agents [12, 13]. It has been found that Cdc5L aberrant

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expression could contribute to variation in cellular proliferation during osteosarcoma pathogenesis, when tumor suppressors RB1, TP53, or the TP53 target CDKN1A is reduced in osteosarcoma [14]. The CDC5L is a potential oncogene in osteosarcoma and cervical tumors, and its depletion has an effect on cell viability [15, 16]. It has been suggested that upregulation of CDC5L is correlated with clinicopathological features and poor prognosis in gliomas, as well as depletion of CDC5L could inhibit proliferation and induce the apoptosis of glioma cells [17]. This study was aimed to evaluate the expressions of MACC1 and CDC5L in patients with osteosarcoma and investigated their associations with clinicopathological parameters.

Materials and methods

Patients and tissues

Paired osteosarcoma tumor tissues and adjacent normal tissues used were collected from 40 patients who underwent operation between 2009 and September 2014 in Tehran, Iran. The tissues were stored at −80 °C until use, and tumor stage was classified according to the TNM classification (Fig. 1). All tissue samples were evaluated by pathologists, and the clinicopathological parameters of all patients were described in Table 1.

RNA extraction and qRT-PCR

Total RNA was extracted from the tissues using miRNeasy kit (Qiagen), according to the manufacturer's protocol. A SYBR Green PCR kit (Qiagen, Germantown, MD) and a Light Cycler 480 instrument (Roche Applied Science) were used for real-time RT-PCR. The relative gene expression was computed relative to phosphoglycerate kinase (PGK) as endogenous control for normalization. The relative amount was evaluated using the comparative cycle threshold (CT) procedure.

Statistical analysis

Differences between expression levels were evaluated using the Student's t test in osteosarcoma tumor tissues and adjacent normal tissues. Relationship between the gene expressions and the clinicopathological parameters were also evaluated by the chi-square test. Differences were considered statistically significant when $P<0.05$.

Results

In the present study, MACC1 and CDC5L mRNA levels in the patients were evaluated using quantitative real-time PCR.

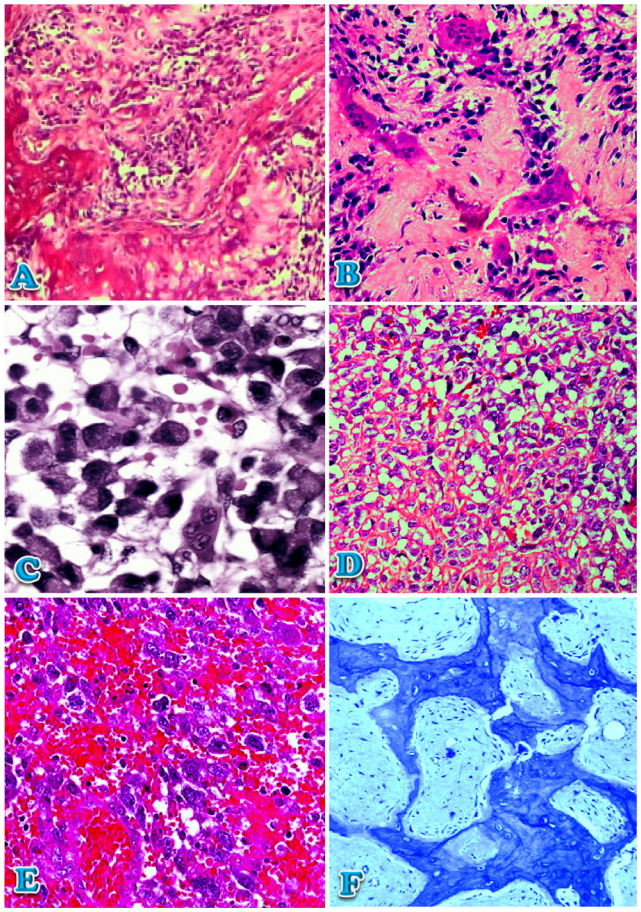

Fig. 1 Photomicrographs of osteosarcoma: consists with neoplastic cells present osteoblastic differentiation and form tumor bone. Some of the figures of the tumor cells are associated with osteoid (H&E)

Our results demonstrated that CDC5L mRNA level was higher in tumor tissues than in adjacent normal tissues (2.713 ± 0.738 vs. 1.071 ± 0.629; $P<0.05$; Fig. 2). Moreover, MACC1 was upregulated in tumor bone tissues than in adjacent normal tissues (3.221 ± 0.624 vs. 1.427 ± 0.456; $P<0.05$; Fig. 3).

According to the median expression level, the expression levels of genes were classified into low and high level. The patients who had the expression level less than median expression were divided as low expression group, and those with high expression were categorized as high expression group.

Correlation of CDC5L and MACC1 expressions with clinicopathological features of osteosarcoma was shown in Table 1. Our results demonstrated that high expression of CDC5L was significantly related to advanced TNM stage ($P=0.032$). No significant difference was determined between CDC5L mRNA expression and other clinicopathological parameters including age, gender, tumor diameter, location, tumor grade, and histological type (Table 1).

In addition, overexpression of MACC1 was strongly correlated with advanced TNM stage ($P=0.027$), and high tumor grade ($P=0.035$). There were no significant differences

Table 1 Correlation of CDC5L and MACC1 expressions with clinicopathological features of osteosarcoma

Clinicopathological features	$N=40$	Expression of CDC5L		Expression of MACC1		P value of CDC5L	P value of MACC1
		Low = 16	High = 24	Low = 13	High = 27		
Gender							
Male	25	9	16	8	17	$P=0.721$	$P=0.623$
Female	15	7	8	5	10		
Age							
≤20 years	18	6	12	7	11	$P=0.522$	$P=0.346$
>20 years	22	10	12	6	16		
Tumor diameter (cm)							
≤5	26	11	15	9	17	$P=0.537$	$P=0.53$
>5	14	5	9	4	10		
Location							
Distal	19	7	12	8	11	$P=0.721$	$P=0.482$
Proximal	21	7	14	5	16		
Tumor grade							
Low	23	7	16	10	13	$P=0.423$	$P=0.035$
High	17	9	8	3	14		
Histological type							
Osteoblastic	16	6	10	5	11	$P=0.715$	$P=0.723$
Chondroblastic	11	4	7	3	8		
Telangiectatic	8	3	5	4	4		
Fibroblastic	5	3	2	1	4		
TNM stage							
I + II	25	11	14	9	16	$P=0.032$	$P=0.027$
III + IV	15	5	10	4	11		

between MACC1 upregulation and other clinicopathological parameters (Table 1).

Discussion

Current evidences indicated that occurrence of osteosarcoma is related to abnormal expression of genes [5]; therefore, the identification of key genes can be helpful for understanding of tumorigenic processes and clarifying the role of new diagnostic, prognostic, and therapeutic markers.

This study was aimed to evaluate the clinical significance of MACC1 and CDC5L in patients with osteosarcoma by using quantitative real-time PCR. Our results demonstrated that CDC5L mRNA expression was higher in tumor tissues than in adjacent normal tissues. The high expression of

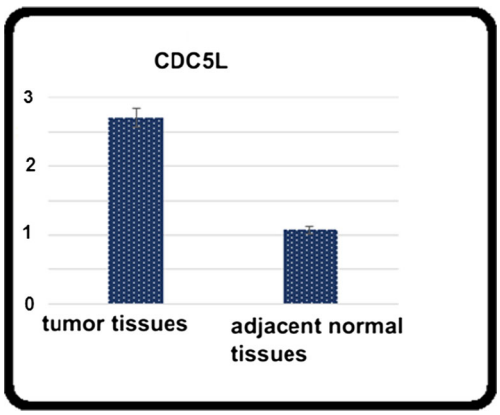

Fig 2 CDC5L expression levels in osteosarcoma and adjacent normal tissues. CDC5L mRNA level was higher in tumor tissues than in adjacent normal tissues ($P<0.05$)

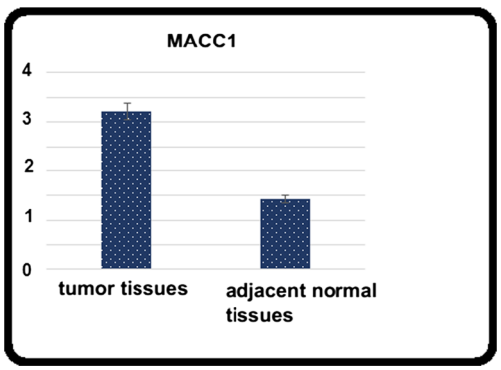

Fig. 3 MACC1 expression levels in osteosarcoma and adjacent normal tissues. MACC1 was upregulated in tumor bone tissues than in adjacent normal tissues ($P<0.05$)

CDC5L was significantly related to advanced TNM stage. No significant difference was determined between CDC5L mRNA expression and other clinicopathological parameters such as age, gender, tumor diameter, location, tumor grade, and histological type. The findings showed that upregulation of CDC5L might be involved in the progression of osteosarcoma. The aberrant expression of CDC5L could contribute to variation in cellular proliferation during osteosarcoma pathogenesis, when tumor suppressors RB1, TP53, or the TP53 target CDKN1A is reduced in osteosarcoma [14]. The CDC5L has been demonstrated to be involved in the occurrence and progression of osteosarcoma and cervical tumors as a potential oncogene, and its depletion has an effect on cell viability [15, 16]. It has been suggested that upregulation of CDC5L is correlated with clinicopathological features and poor prognosis in gliomas, as well as depletion of CDC5L could inhibit proliferation and induce the apoptosis of glioma cells [17]. Previous studies indicated that CDC5L is an important cell cycle regulator for the G2-M transition, and its overexpression of CDC5L can lead to shorter G2/M cell cycle transition [15], as well as upregulation of CDC5L could be involved in cellular proliferation during osteosarcoma pathogenesis [14]. However, further studies are required to clarify the role CDC5L in patients with osteosarcoma. On the other hand, MACC1 was upregulated in tumor bone tissues when compared with adjacent normal tissues. Moreover, upregulation of MACC1 was strongly correlated with advanced TNM stage and higher tumor grade. There were no significant differences between MACC1 upregulation and other clinicopathological parameters. Overexpression of MACC1 has been found in many kinds of tumors, including gastric carcinoma, breast carcinoma, ovarian cancer, and hepatocellular carcinoma [6–8]. Furthermore, higher expression of MACC1 has been shown to be markedly correlated with poor prognosis and metastasis in many kinds of malignancies, such as gastric carcinoma, esophageal cancer, lung cancer, and colon cancer [9–11]. Shirahata et al. [11] evaluated the expression levels of MACC1 in gastric cancer patients using qRT-PCR. They indicated that upregulation of MACC1 is correlated with clinicopathological parameters in gastric cancer and might serve as a new marker for the prognostic prediction. Furthermore, MACC1 overexpression has been reported in many kinds of tumor types and can serve as an important marker of poor prognosis and metastasis [18–20]. Jin et al. [21] found that MACC1 expression was correlated with TNM stage, distant metastasis, and renal cell carcinoma (RCC) prognosis. This indicated that high MACC1 expression is related to RCC aggressiveness and may be involved in development of renal cell carcinoma. Zhang et al. demonstrated that high MACC1 level was strongly associated with clinical stage, distant metastasis, and poor survival in patients with osteosarcoma [22]. Previous findings are more or less in agreement with our results on the different expressions of the biomarkers in human osteosarcoma [23–25]. However, further investigation is needed to evaluate the role of these markers and their involved mechanisms in the development of osteosarcoma.

In conclusion, our findings indicated that mRNA overexpression of MACC1 was strongly correlated with advanced TNM stage and high tumor grade, as well as CDC5L expression were correlated with advanced TNM stage, and MACC1 may be involved in development and progression of osteosarcoma.

Compliance with ethical standards

Conflicts interest None

References

1. Broadhead ML, Clark JC, Myers DE, Dass CR, Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma. 2011;2011:959248.
2. Jaffe N. Osteosarcoma: review of the past, impact on the future. The American experience. Cancer Treat Res. 2009;152:239–62.
3. Thayanithy V, Park C, Sarver AL, Kartha RV, Korpela DM, Graef AJ. Combinatorial treatment of DNA and chromatin-modifying drugs cause cell death in human and canine osteosarcoma cell lines. PLoS ONE. 2012;7(9):e43720.
4. Hu H, Zhang Y, Cai XH, Huang JF, Cai L. Changes in microRNA expression in the MG-63 osteosarcoma cell line compared with osteoblasts. Oncol Lett. 2012;4(5):1037–42.
5. Liu LH, Li H, Li JP, Zhong H, Zhang HC, Chen J. Xiao T.miR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3. Biochem Biophys Res Commun. 2011;416(1–2):31–8.
6. Huang Y, Zhang H, Cai J, Fang L, Wu J, Ye C. Overexpression of MACC1 and Its significance in human breast cancer progression. Cell Biosci. 2013;3(1):16.
7. Gao J, Ding F, Liu Q, Yao Y. Knockdown of MACC1 expression suppressed hepatocellular carcinoma cell migration and invasion and inhibited expression of MMP2 and MMP9. Mol Cell Biochem. 2013;376(1–2):21–32.
8. Qu JH, Chang XJ, Lu YY, Bai WL, Chen Y, Zhou L, et al. Overexpression of metastasis-associated in colon cancer 1 predicts a poor outcome of hepatitis B virus-related hepatocellular carcinoma. World J Gastroenterol. 2012;18(23):2995–3003.
9. Shimokawa H, Uramoto H, Onitsuka T, Chundong G, Hanagiri T, Oyama T. Overexpression of MACC1 mRNA in lung adenocarcinoma is associated with postoperative recurrence. J Thorac Cardiovasc Surg. 2011;141(4):895–8.
10. Shirahata A, Shinmura K, Kitamura Y, Sakuraba K, Yokomizo K, Goto T. MACC1 as a marker for advanced colorectal carcinoma. Anticancer Res. 2010;30(7):2689–92.
11. Shirahata A, Sakata M, Kitamura Y, Sakuraba K, Yokomizo K, Goto T. MACC 1 as a marker for peritoneal-disseminated gastric carcinoma. Anticancer Res. 2010;30(9):3441–4.
12. Bernstein HS. Coughlin SR.A mammalian homolog of fission yeast Cdc5 regulates G2 progression and mitotic entry. J Biol Chem. 1998;273(8):4666–71.
13. Zhang N, Kaur R, Akhter S, Legerski RJ. Cdc5L interacts with ATR and is required for the S-phase cell-cycle checkpoint. EMBO Rep. 2009;10(9):1029–35.

14. Martin JW, Chilton-MacNeill S, Koti M, van Wijnen AJ, Squire JA, Zielenska M. Digital expression profiling identifies RUNX2, CDC5L, MDM2, RECQL4, and CDK4 as potential predictive biomarkers for neo-adjuvant chemotherapy response in paediatric osteosarcoma. PLoS ONE. 2014;9(5):e95843.
15. Mu R, Wang YB, Wu M, Yang Y, Song W, Li T. Depletion of pre-mRNA splicing factor Cdc5L inhibits mitotic progression and triggers mitotic catastrophe. Cell Death Dis. 2014;5:e1151.
16. Lu XY, Lu Y, Zhao YJ, Jaeweon K, Kang J, Xiao-Nan L. Cell cycle regulator gene CDC5L, a potential target for 6p12-p21 amplicon in osteosarcoma. Mol Cancer Res. 2008;6(6):937–46.
17. Chen W, Zhang L, Wang Y, Sun J, Wang D, Fan S, et al. Expression of CDC5L is associated with tumor progression in gliomas. Tumour Biol. 2015. doi:10.1007/s13277-015-4088-5
18. Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, et al. MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med. 2009;15:59–67.
19. Janowitz T, Welsh SJ, Zaki K, Mulders P, Eisen T. Adjuvant therapy in renal cell carcinoma-past, present, and future. Semin Oncol. 2013;40:482–91.
20. Arlt F, Stein U. Colon cancer metastasis: MACC1 and Met as metastatic pacemakers. Int J Biochem Cell Biol. 2009;41:2356–9.
21. Jin Z, Xu N, Guo K. Increased expression of metastasis-associated in colon cancer-1 in renal cell carcinoma is associated with poor prognosis. Int J Clin Exp Pathol. 2015;8(4):3857–63.
22. Zhang K, Zhang Y, Zhu H, Xue N, Liu J, Shan C, et al. High expression of MACC1 predicts poor prognosis in patients with osteosarcoma. Tumour Biol. 2014;35(2):1343–50.
23. Taheriazam A, Bahador R, Karbasy SH, Jamshidi SM, Torkaman A, Yahaghi E, et al. Down-regulation of microRNA-26a and up-regulation of microRNA-27a contributes to aggressive progression of human osteosarcoma. Diagn Pathol. 2015;10:166.
24. Esmailiejah AA, Taheriazam A, Golbakhsh MR, Jamshidi M, Shakeri M, Yahaghi E, et al. Analysis of serum levels and tissue expression of galectin-1 and galectin-3 as noninvasive biomarkers in osteosarcoma patients. Tumour Biol. 2015.
25. Taheriazam A, Talaei AJ, Jamshidi M, Shakeri M, Khoshbakht S, Yahaghi E, et al. Up-regulation of miR-130b expression level and down-regulation of miR-218 serve as potential biomarker in the early detection of human osteosarcoma. Diagn Pathol. 2015;10:184.