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Original Article

Association of matrix metalloproteinase-1 polymorphism with the risk and prognosis of osteosarcoma patients

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Abstract: Aim: Matrix metalloproteinase-1 (*MMP-1*) polymorphisms are related to elevated susceptibility of tumor development and poor prognosis of patients in several cancers. The current study aimed to determine whether *MMP-1* polymorphisms were associated with the risk and prognosis of osteosarcoma patients. Methods: *MMP-1*-1607 1G/2G genotype variants were identified using denaturing high performance liquid chromatography (DHPLC) and DNA sequencing techniques. χ^2 test was used to evaluate the association of the polymorphisms and clinical characteristics. Results: The Kaplan-Meier and Cox regression analysis were performed to detect the prognostic values of *MMP-1* polymorphisms. Our findings exhibited that 1G/2G (OR=4.219, 95% CI=2.040-8.728, *P*<0.05) and 2G/2G (OR=8.770, 95% CI=3.657-21.030, *P*<0.05) variants presented more frequently in osteosarcoma patients while 1G/1G was less common in osteosarcoma patients. Moreover, there was significant association between *MMP-1*-1607 1G/2G genotype and clinicopathologic characteristics including Enneking stages, distant metastasis, response to chemotherapy and recurrence while no relations were found in gender and age. Conclusions: Osteosarcoma patients with 1G/2G genotype and 2G/2G. Genotype presented a shorter survival time compared with those with 1G/1G via Kaplan-Meier analysis (*P*=0.000). Cox regression analysis suggested that *MMP-1*-1607 1G/2G (HR=3.657, 95% CI=1.020-13.109, *P*=0.047) and 2G/2G (HR=12.028, 95% CI=3.089-46.861, *P*=0.000) genotypes and recurrence (HR=2.513, 95% CI=1.253-5.040, *P*=0.009) might be potential prognostic markers of osteosarcoma patients. Taken together, our studies demonstrated that *MMP-1* polymorphisms was related with the risk of osteosarcoma and possessed important prognostic value.

Keywords: Osteosarcoma, polymorphism, *MMP-1*-1607 1G/2G, prognosis

Introduction

Osteosarcoma (OS) is one of the most common primary bone malignancy and mostly appears in the blood circulation-abundant metaphyseal of long bone including distal femur and proximal tibia [1, 2]. It accounts for 5% of childhood cancers and 8.9% of cancer-related deaths in children [3]. Osteosarcoma is known as the disease resulted from tumor tissue erosion and bone cortex dissolution in tumor site. As it has a high rate of metastasis, the 5-year survival rate and the cure rate are both very low. With the development of comprehensive therapies, the 5-year survival rate has raised to 60-70% [4, 5]. However, improvements in osteosarcoma survival during the last decade have been limited and the patients with osteosarcoma are still with a high mortality [6]. It is difficult to ultimately heal osteosarcoma through traditional treatment thus a large portion of sufferers still present local relapse or distant metastasis after operation or chemotherapy [7, 8]. Clearly, new treatment strategies are needed and it is of great significance to identify and screen novel tumor markers to predict osteosarcoma.

Matrix metalloproteinase-1 (*MMP-1*), a member of MMP family, is an endogenous peptide enzyme which most widely expresses in interstitial collagenase that can degrade extracellular matrix. It is involved in many stages of tumorigenesis such as promote tumor growth via stimulating cellular proliferation, invasion

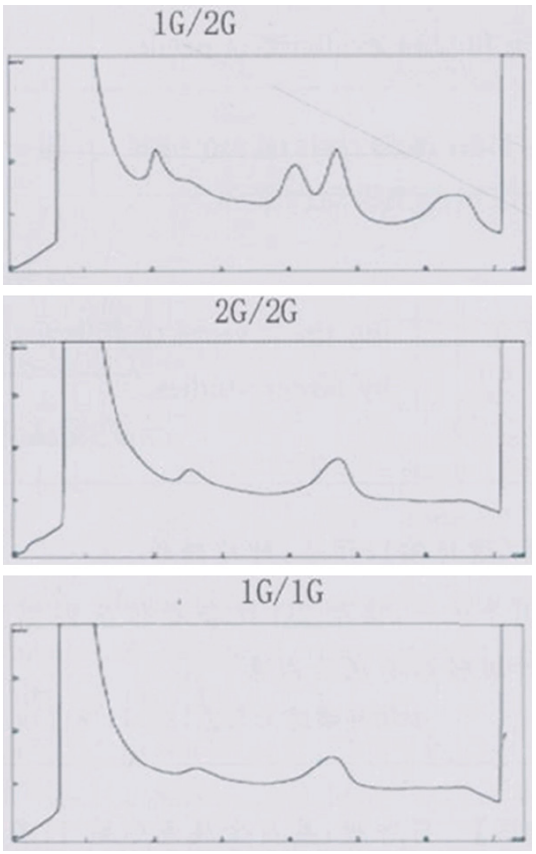

Figure 1. Denaturing high performance liquid chromatography (DHPLC) was performed to determine *MMP-1*-1607 1G/2G genotype. 1G/2G genotype appeared double peaks in DHPLC. Analysis displayed that single peak of 2G/2G genotype and 1G/1G genotype occurred after equally mixing the two PCR samples.

and migration, angiogenesis, and suppressing tumor cells apoptosis [9, 10]. *MMP1*-1607 1G/2G (rs1799750) contains a guanine insertion/deletion polymorphism at position-1607 and is a functional single nucleotide polymorphism (SNP) that can up-regulate the expression of *MMP1* [11, 12]. The association between the *MMP1*-1607 1G/2G polymorphism and the emergence and development of several diseases including the risk of many cancers has been reported [11-18]. Besides, many studies have confirmed that *MMP-1*-1607 1G/2G polymorphism also has an important prognostic value. However, the effects of *MMP-1*-1607 1G/2G polymorphism on osteosarcoma remains unclear.

In this study, we aimed to investigate the genotype of *MMP-1* polymorphism and explore the prognostic value of different genotypes in

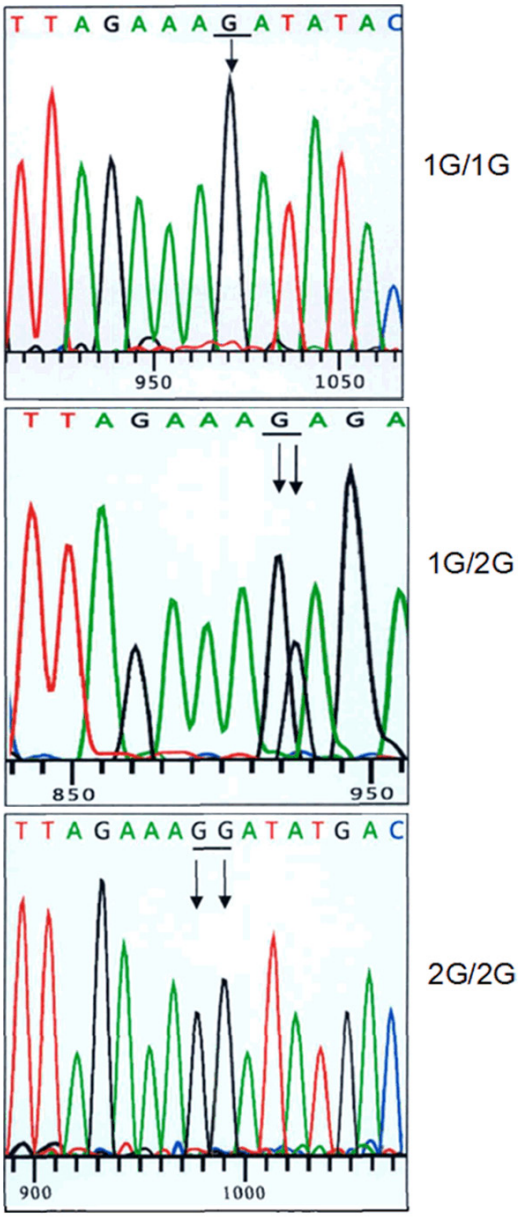

Figure 2. DNA sequences of *MMP-1* gene in 110 osteosarcoma patients and 80 healthy individuals were obtained using direct sequencing machine. One G base insertion caused polymorphism of *MMP-1*-1607 1G/2G including 1G/1G, 1G/2G and 2G/2G genotypes.

osteosarcoma patients. The research results were expected to provide a new strategy for the therapy of osteosarcoma.

Materials and methods

Case and control samples

A total of 110 osteosarcoma patients and 80 healthy individuals were collected in the study

Table 1. The genotypes of *MMP-1*-1607 1G/2G in osteosarcoma patients

Genotype	OS group	Control group	OR (95% CI)	*P*
N	110	80		
1G/1G	17	40	1.000	-
1G/2G	52	29	4.219 (2.040-8.728)	*P*<0.05
2G/2G	41	11	8.770 (3.657-21.030)	*P*<0.05

from May 12, 2009 to March 10, 2012. And the study was conducted in The First Affiliated Hospital of Chongqing Medical University and permitted by the Ethic Committee of the hospital. All participates had signed informed consents in advance.

The tumor tissues and healthy tissues were obtained from the patients and controls, respectively. Then the tissues were frozen in liquid nitrogen quickly and stored at -80°C for the following analysis. Besides a 5-year follow-up was conducted with the osteosarcoma patients. The clinical characteristics including age, gender, Enneking stages, distant metastasis, response to chemotherapy and recurrence were recorded in a database. Patients who died from unexpected events or other diseases were excluded from our study.

DNA extraction and PCR amplification

The QIAamp DNA Mini Kit (Qiagen) was used to extract DNA from the patients and controls according to the proposals of manufacturer. The PCR amplification was conducted by AmpℓFSTR® Identifier® Plus PCR Amplification Kit (Life Technologies). The PCR reaction mixture included 2.5 μl of 10× Ex Taq, 2 μl dNTP, 0.3 μl forward-primer and 0.3 μl reverse-primer, 2 μl DNA, 0.2 μl Ex Taq and 17.7 μl ddH_2O. The reaction procedures were as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation for 40 s at 94°C, annealing at 56°C for 40 s, extension at 72°C for 40 s, and finally extension at 72°C for 10 min. The PCR products were preserved at 4°C.

Determination of the genotype

Denaturing high performance liquid chromatography (DHPLC) was performed to detect the genotype of the *MMP-1*-1607 1G/2G using the WAVE system (Transgenomic, USA). Double peaks were considered to be 1G/2G heterozygous genotype, while single peak was considered as 1G/1G and 2G/2G genotype respectively, according to different retention time. Moreover, direct DNA sequencing was used to verify the outcome of DHPLC. The direct sequencing of DNA was conducted in ABI 3730 sequenator (Applied Biosystems).

Statistical analysis

A value of $P<0.05$ was considered to be statistically significant. The statistical analyses were carried out in the SPSS 20.0 software (SPSS Inc., Chicago, USA). χ^2 test was performed to assess the variances in genotype distributions among groups and the association of genotype and clinical characteristics, respectively. Besides, the relationship between genotypes of *MMP-1* and the overall survival was explored via Kaplan-Meier analysis while the prognostic value was estimated by Cox regression.

Results

Identification and distribution of genotypes

DHPLC and DNA sequencing were used to determine *MMP-1*-1607 1G/2G genotype (**Figures 1** and **2**). As displayed in **Table 1**. Genotype frequency of polymorphism site *MMP-1*-1607 1G/2G in healthy individuals was agreed with Hardy-Weinberg (χ^2=2.186, P=0.139), suggesting samples were with good representative. The distribution of *MMP-1*-1607 1G/2G genotype in osteosarcoma patients was significantly different from that in control group. The risk of osteosarcoma patients carrying 1G/2G (OR=4.219, 95% CI=2.040-8.728, $P<0.05$) and 2G/2G (OR=8.770, 95% CI=3.657-21.030, $P<0.05$) genotypes was higher than those carrying 1G/1G genotype.

Relationship between MMP-1-1607 1G/2G and clinical characteristics in osteosarcoma

According to the genotypes, 110 osteosarcoma patients were divided into three groups including 1G/1G (15.46%) group, 1G/2G (47.27%) group and 2G/2G (37.27%) group. The results of χ^2 test showed that *MMP-1*-1607 1G/2G polymorphism was influenced by Enneking stages (χ^2=12.858, P=0.012), distant metastasis (χ^2=21.998, P=0.000), response to chemotherapy (χ^2=7.794, P=0.020) and recurrence (χ^2=6.110, P=0.047) significantly (**Table 2**).

Table 2. Association of *MMP-1*-1607 1G/2G and clinical characteristics in patients with osteosarcoma

Parameters	N	Genotype			χ^2	*P*
		1G/1G	1G/2G	2G/2G		
Total	110	17	52	41		
Gender					4.391	0.111
Male	96	13	44	39		
Female	14	4	8	2		
Age					1.117	0.572
≤ 20	58	10	29	19		
> 20	52	7	23	22		
Enneking stages					12.858	0.012
I	22	3	12	7		
II	19	7	10	2		
III	69	7	30	32		
Distant metastasis					21.998	0.000
Positive	99	10	49	40		
Negative	11	7	3	1		
Response to chemotherapy					7.794	0.020
Poor	86	9	42	35		
Good	24	8	10	6		
Recurrence					6.110	0.047
Positive	79	8	40	31		
Negative	31	9	12	10		

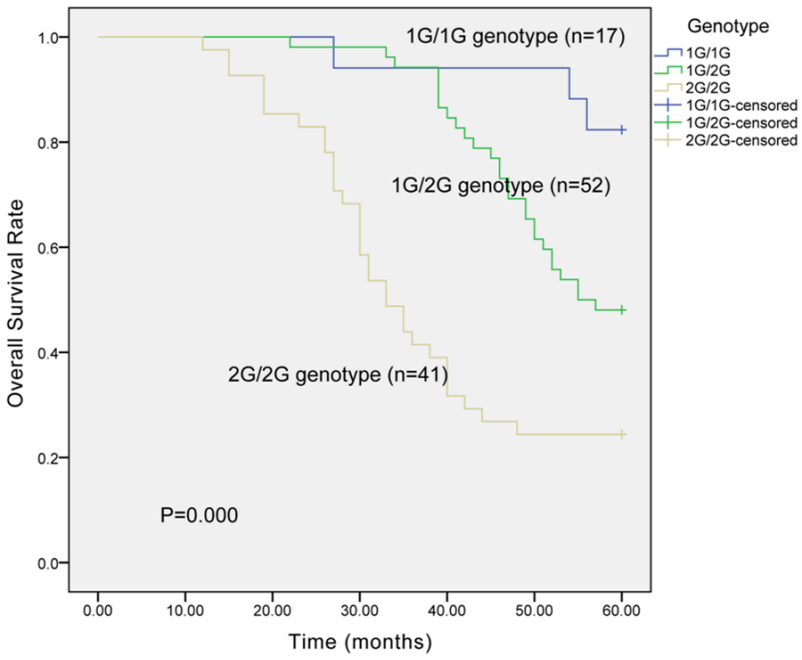

Figure 3. Kaplan-Meier analysis showed a shorter survival time of patients with 1G/2G and 2G/2G genotypes than those with 1G/1G genotype (*P*=0.000).

However, no correlations were observed between genotype of *MMP-1*-1607 1G/2G with gender (χ^2=4.391; *P*=0.111) and age (χ^2=1.117, *P*=0.572) in patients.

Prognostic value of MMP-1-1607 1G/2G polymorphism in osteosarcoma patients

Association between the overall survival and different genotypes of *MMP-1* was analyzed by Kaplan-Meier analysis and tested via log rank test. The result demonstrated that patients with *MMP-1*-1607 1G/2G and 2G/2G genotype lived longer than those with 1G/1G genotype. Besides, the patients with 1G/2G genotype lived longer than patients with 2G/2G genotype (**Figure 3**, *P*=0.000). Cox regression analysis showed that recurrence (HR=2.513, 95% CI=1.253-5.040, *P*=0.009) and the mutant genotypes (1G/2G and 2G/2G) (HR=3.657, 95% CI=1.020-13.109, *P*=0.047 and HR=12.028, 95% CI=3.089-46.861, *P*=0.000, respectively) were all influencing factors in the prognosis for osteosarcoma patients (**Table 3**). And they might be promising prognostic markers in patients with osteosarcoma.

Discussion

Osteosarcoma is a differentiation disease which is caused by genetic changes that interrupt osteoblast differentiation from mesenchymal stem cells [19]. As the easy relapse of osteosarcoma, the prognosis of this disease is very poor and the cure rate is less than 65% for localized osteosarcoma patients [20]. So prognostic markers are impor-

Table 3. Multivariable Cox regression assay of osteosarcoma indicators

Characteristics	HR	95% CI	*P*
Gender	0.761	0.312-10854	0.548
Metastasis	1.139	0.392-3.313	0.8111
Response to chemotherapy	1.233	0.591-2.574	0.577
Recurrence	2.513	1.253-5.040	0.009
1G/1G	1.000	-	-
1G/2G	3.657	1.020-13.109	0.047
2G/2G	12.028	3.089-46.861	0.000

tant to osteosarcoma. Moreover, some common markers (including miRNAs, lncRNAs, and gene polymorphism) which predict the prognosis of osteosarcoma have been found recent years. In previous studies, there are many reports about the relationship between gene polymorphism and osteosarcoma. For instance, Jiang C et al. have found that rs1906953 in the glutamate receptor metabotropic 4 (GRM4) gene was not only associated with the metastasis and prognosis but also related to the risk of osteosarcoma which indicated that GRM4 polymorphism was a susceptive and prognostic factor in osteosarcoma [21]. Rho GTPase-activating protein 35 rs1052667 polymorphism was studied and confirmed to be an independent prognostic marker as well as a stimulative for the occurrence of osteosarcoma [22].

MMP-1 locates in the 11q22 of human chromosome and contains 10 exons and 9 introns. It usually expresses at low levels in most normal cells, while its expression reaches high levels both in tumor cells and stromal cells, which was closely related to tumorous local infiltrating growth and distant metastasis. Moreover, obvious association between prognosis and *MMP-1* gene was also discovered in some cancers including melanoma, ovarian cancer, endometrial cancer and gastric cancer [23, 24]. 1G/2G polymorphism lied in *MMP-1* promoter -1607 site could produce 5'-GGAT-3' site bonding with Ets via G base insertion, and then drive *MMP-1* gene transcription and up-regulate expression [25]. *MMP-1*-1607 1G/2G polymorphism was verified to be linked with the risk of various diseases such as oral squamous cell carcinoma, nasopharyngeal carcinomas, periodontitis, chronic pancreatitis, and primary knee osteoarthritis [13, 26-29]. However, up to now the research about the relationship between *MMP-1*-1607 1G/2G polymorphism and osteosarcoma is very scarce.

Our study gathered tissue samples from 110 osteosarcoma patients and 80 normal subjects, extracted their DNA and detected the polymorphism site of *MMP-1*-1607 1G/2G, thus explored the correlation of *MMP-1*-1607 1G/2G polymorphism and osteosarcoma. The results showed that significant difference of *MMP-1*-1607 1G/2G genotype distribution occurred in patients with osteosarcoma and healthy controls. It was more easily to suffer from osteosarcoma for people with 1G/2G and 2G/2G genotypes compared to those with 1G/1G genotype which manifested the closely association between *MMP-1*-1607 1G/2G polymorphism and the risk of osteosarcoma.

In addition, according to the study we found that *MMP-1*-1607 1G/2G polymorphism was impacted by several clinical characteristics including Enneking stage, distant metastasis, response to chemotherapy and recurrence. It indicated that *MMP-1*-1607 1G/2G polymorphism might take effects on the development process of osteosarcoma. To further analyze the prognostic value of *MMP-1*-1607 1G/2G polymorphism, Kaplan-Meier and Cox regression analysis were conducted. The result of Kaplan-Meier showed the survival time of osteosarcoma patients with genotype of 1G/1G was longer than patients with genotypes of 1G/2G and 2G/2G. Meanwhile, Cox regression analysis revealed that *MMP-1*-1607 1G/2G polymorphism as well as recurrence was vital prognostic factors in osteosarcoma patients. And they might be independent prognostic markers.

In conclusion, our results have provided full proofs to validate that *MMP-1* polymorphism can predict the munity and prognosis of osteosarcoma. *MMP-1*-1607 1G/2G genotype and 2G/2G genotype might be potential prognostic markers for osteosarcoma patients. These results are consistent with the previous studies and support the hypothesis that aberrant *MMP-1* expression may relate with tumor progression. Whereas, the molecular mechanism and signal pathways about *MMP-1*-1607 1G/2G polymorphism in osteosarcoma still needs further study.

Disclosure of conflict of interest

None.

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References

[1] Bramwell VH. Osteosarcomas and other cancers of bone. Curr Opin Oncol 2000; 12: 330-336.

[2] Siegel HJ and Pressey JG. Current concepts on the surgical and medical management of osteosarcoma. Expert Rev Anticancer Ther 2008; 8: 1257-1269.

[3] Yang J and Zhang W. New molecular insights into osteosarcoma targeted therapy. Curr Opin Oncol 2013; 25: 398-406.

[4] Mirabello L, Troisi RJ and Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1531-1543.

[5] Broadhead ML, Clark JC, Myers DE, Dass CR and Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma 2011; 2011: 959248.

[6] Meng Q, Zhao J, Liu H, Zhou G, Zhang W, Xu X and Zheng M. HMGB1 promotes cellular proliferation and invasion, suppresses cellular apoptosis in osteosarcoma. Tumour Biol 2014; 35: 12265-12274.

[7] Kansara M and Thomas DM. Molecular pathogenesis of osteosarcoma. DNA Cell Biol 2007; 26: 1-18.

[8] Yan J, Wang Q, Zou K, Wang L, Schwartz EB, Fuchs JR, Zheng Z and Wu J. Inhibition of the JAK2/STAT3 signaling pathway exerts a therapeutic effect on osteosarcoma. Mol Med Rep 2015; 12: 498-502.

[9] Efendioglu M, Basaran R, Bayrak OF, Isik N, Kaner T, Sahin F, Guclu B and Elmaci I. The role of a single nucleotide polymorphism of the matrix metalloproteinase-1 gene promoter region in invasion and prognosis of meningiomas. Turk Neurosurg 2014; 24: 731-736.

[10] Fink K and Boratynski J. [The role of metalloproteinases in modification of extracellular matrix in invasive tumor growth, metastasis and angiogenesis]. Postepy Hig Med Dosw (Online) 2012; 66: 609-628.

[11] Zhang C, Song X, Zhu M, Shi S, Li M, Jin L, Lang J, Li G and Zheng H. Association between MMP1 -1607 1G>2G polymorphism and head and neck cancer risk: a meta-analysis. PLoS One 2013; 8: e56294.

[12] Fakhoury H, Noureddine S, Chmaisse HN, Tamim H and Makki RF. MMP1-1607(1G>2G) polymorphism and the risk of lung cancer in Lebanon. Ann Thorac Med 2012; 7: 130-132.

[13] Nasr HB, Mestiri S, Chahed K, Bouaouina N, Gabbouj S, Jalbout M and Chouchane L. Matrix metalloproteinase-1 (-1607) 1G/2G and -9 (-1562) C/T promoter polymorphisms: susceptibility and prognostic implications in nasopharyngeal carcinomas. Clin Chim Acta 2007; 384: 57-63.

[14] Mutlag AM, Wang X, Yang Z, Meng J, Zhang J, Qin Z, Wang G and Li J. Study on matrix metalloproteinase 1 and 2 gene expression and NO in dairy cows with ovarian cysts. Anim Reprod Sci 2015; 152: 1-7.

[15] Dey S, Ghosh N, Saha D, Kesh K, Gupta A and Swarnakar S. Matrix metalloproteinase-1 (MMP-1) Promoter polymorphisms are well linked with lower stomach tumor formation in eastern Indian population. PLoS One 2014; 9: e88040.

[16] Olejniczak I, Fendler W, Borowiec M, Mlynarski W and Pietruszewska W. [Polymorphism of metalloproteinases MMP-1 and MMP-2 in risk of laryngeal cancer]. Otolaryngol Pol 2012; 66: 30-35.

[17] Albayrak S, Canguven O, Goktas C, Aydemir H and Koksal V. Role of MMP-1 1G/2G promoter gene polymorphism on the development of prostate cancer in the Turkish population. Urol Int 2007; 79: 312-315.

[18] Matsumura S, Oue N, Kitadai Y, Chayama K, Yoshida K, Yamaguchi Y, Toge T, Imai K, Nakachi K and Yasui W. A single nucleotide polymorphism in the MMP-1 promoter is correlated with histological differentiation of gastric cancer. J Cancer Res Clin Oncol 2004; 130: 259-265.

[19] Tang N, Song WX, Luo J, Haydon RC and He TC. Osteosarcoma development and stem cell differentiation. Clin Orthop Relat Res 2008; 466: 2114-2130.

[20] Song QC, Shi ZB, Zhang YT, Ji L, Wang KZ, Duan DP and Dang XQ. Downregulation of microRNA-26a is associated with metastatic potential and the poor prognosis of osteosarcoma patients. Oncol Rep 2014; 31: 1263-1270.

[21] Jiang C, Chen H, Shao L and Dong Y. GRM4 gene polymorphism is associated with susceptibility and prognosis of osteosarcoma in a Chinese Han population. Med Oncol 2014; 31: 50.

[22] Zhao J, Xu H, He M, Wang Z and Wu Y. Rho GTPase-activating protein 35 rs1052667 polymorphism and osteosarcoma risk and prognosis. Biomed Res Int 2014; 2014: 396947.

[23] Brinckerhoff CE, Rutter JL and Benbow U. Interstitial collagenases as markers of tumor progression. Clin Cancer Res 2000; 6: 4823-4830.

[24] Inoue T, Yashiro M, Nishimura S, Maeda K, Sawada T, Ogawa Y, Sowa M and Chung KH.

Matrix metalloproteinase-1 expression is a prognostic factor for patients with advanced gastric cancer. Int J Mol Med 1999; 4: 73-77.

[25] Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1998; 58: 5321-5325.

[26] Vairaktaris E, Yapijakis C, Derka S, Serefoglou Z, Vassiliou S, Nkenke E, Ragos V, Vylliotis A, Spyridonidou S, Tsigris C, Yannopoulos A, Tesseromatis C, Neukam FW and Patsouris E. Association of matrix metalloproteinase-1 (-1607 1G/2G) polymorphism with increased risk for oral squamous cell carcinoma. Anticancer Res 2007; 27: 459-464.

[27] Ustun K, Alptekin NO, Hakki SS and Hakki EE. Investigation of matrix metalloproteinase-1--1607 1G/2G polymorphism in a Turkish population with periodontitis. J Clin Periodontol 2008; 35: 1013-1019.

[28] Sri Manjari K, Nallari P, Balakrishna N, Vidyasagar A, Prabhakar B, Jyothy A and Venkateshwari A. Influence of matrix metalloproteinase-1 gene -1607 (1G/2G) (rs1799750) promoter polymorphism on circulating levels of MMP-1 in chronic pancreatitis. Biochem Genet 2013; 51: 644-654.

[29] Lepetsos P, Pampanos A, Kanavakis E, Tzetis M, Korres D, Papavassiliou AG and Efstathopoulos N. Association of MMP-1 -1607 1G/2G (rs1799750) polymorphism with primary knee osteoarthritis in the Greek population. J Orthop Res 2014; 32: 1155-1160.