Original article

The relationship between MDM2 expression and tumor thickness and invasion in primary cutaneous malignant melanoma

Parvin Rajabi[1], Paridokht Karimian[2], Mitra Heidarpour[3]

[1]Professor, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. [2]Resident, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. [3]Associate Professor, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Malignant melanoma is the most invasive cutaneous tumor which is associated with an incredibly high mortality rate. The most reliable histological factors associated with melanoma prognosis are tumor thickness- measured by the Breslow index- and invasion depth- measured by Clark level. Murine double minute 2 (MDM2) gene inhibits p53-dependent apoptosis. An increase in MDM2 expression has been found in many tumors. This study aimed to investigate MDM2 expression and its correlation with tumor thickness and invasion level in malignant melanoma. **Materials and Methods:** This study evaluated paraffin blocks from 43 randomly selected patients with primary cutaneous melanoma who referred to the main university pathology center in Isfahan, Iran. MDM2 expression rate was assessed via immunohistochemical techniques and hematoxylin and eosin staining to determine tumor thickness and invasion level. Correlations between MDM2 expression and tumor thickness and invasion were analyzed using Spearman's correlation coefficient in $SPSS_{17}$. **Results:** The mean age of patients was 61.2 ± 15 years. Men and women constituted 55.8% and 44.2% of the participants, respectively. The rate of MDM2 positivity was 28.9%. MDM2 expression was directly associated with tumor thickness ($r = 0.425$; $p = 0.002$) and weakly with invasion level ($r = 0.343$; $p = 0.01$). **Conclusions:** Despite the low MDM2 expression rate observed in this study, direct relationships between MDM2 positivity and tumor thickness and invasion level were identified. MDM2 expression can thus be suggested as a potential new predictive prognostic factor.

Key words: Melanoma, Murine Double Minute 2, Prognosis.

INTRODUCTION

The incidence of melanoma has continued to increase worldwide. Incidence rates vary with respect to age and gender. While melanoma is more common in women than in men prior to 50 years of age, this trend reverses after age 50.[1-5] Although melanoma accounts for roughly 4% of all skin cancers, it is responsible for more than 74% of skin cancer deaths.[6]

Tumor thickness, as defined by the Breslow index, is the most important histological prognostic factor of melanoma and is measured vertically in millimeters from the top of the granular layer (or base of superficial ulceration) to the deepest point of tumoral involvement. Increased tumor thickness suggests a higher metastatic potential and a poorer prognosis.[3,4] The Clark level on the other hand, is a measure of the anatomical invasion of the tumor and appears to affect prognosis only in thinner (<1 mm of depth) melanomas.[5]

Murine double minute 2 (MDM2) is a 491 amino acid protein that functions as a key regulator of P53 by binding to its N-terminus, inhibiting its transcriptional activity, and promoting its degradation.[7,8] MDM2 overexpression or hyperactivation directly contributes to loss of p53 function during the development of nearly 50% of human cancers.[9] Amplification of the genetic locus of MDM2 is a common event in several tumors including glioma, liposarcoma, osteosarcoma, pancreatic ductal adenocarcinoma, and germ cell tumors.[10-14] The MDM2 immunoreactivity of tumoral cells has also been shown to be potentially correlated with the outcome.[15,16] A strong correlation between MDM2 expression and progressive disease has also been demonstrated.[17,18] Most previous studies have used polymerase chain reaction (PCR) for their analyses. However, we used immunohistochemical techniques because finding a significant correlation between MDM2 expression and tumor thickness and invasion would introduce a predictive prognostic marker for cutaneous malignant melanoma. Therefore, the relationship between MDM2 expression and tumor thickness and invasion in primary cutaneous malignant melanoma was evaluated.

Address for correspondence: Mitra Heidarpour, Associate Professor, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Email: heidarpour@med.mui.ac.ir
Received: 28-04-2012; **Revised:** 10-05-2012; **Accepted:** 28-05-2012

MATERIALS AND METHODS

This was a descriptive analytical study on specimens from 43 randomly selected patients with primary cutaneous malignant melanoma who had undergone surgery or biopsy at Alzahra Hospital (Isfahan, Iran) during 2005-11.

The study included 24 men and 19 women who aged 32-89 years. All paraffin blocks of the patients were studied and the most appropriate ones were selected. Afterwards, 2 slides were made from each block to be either stained with hematoxylin and eosin, or subjected to immunohistochemical analysis using a mouse monoclonal MDM2 antibody (NCL-MDM2, Novocastra). Based on manufacturer instructions, immunohistochemical staining was performed using the following stages: 1) H_2O_2 (hydrogen peroxide) stage; 2) incubation of the tissue with the primary MDM2 antibody for 30 minutes; 3) incubation with polymer (EnVision; secondary antibody) for 30 minutes; and 4) incubation with diaminobenzidine (DAB) chromogen substrate solution for 5 minutes. At every stage, after the application of the above-mentioned materials, the slides were washed with a buffer solution or distilled water. For further staining, hematoxylin was employed and finally copper sulfate 0.5% was applied for two minutes. Next, the slides stained with MDM2 were assessed. The percentage of immunoreactive nuclei was evaluated by counting at least 500 cells using the ×40 objective lens.[18,19] Positive controls included cases of colon carcinoma. Negative controls were obtained by omitting the primary monoclonal antibody.

The stained slides were scored based on stain intensity and stainability. The scores were defined as positive when there was nuclear staining in at least 10% of neoplastic cells. Such slides were categorized as weak, moderate, or strong based on their intensity.[18,19]

Associations between the intensity of MDM2 expression and the depth of invasion (Clark level) and tumor thickness (according to Breslow index) were evaluated using Spearman's correlation coefficients. The level of significance was set at $p < 0.05$. $SPSS_{17}$ was used for analyses.

RESULTS

Of 43 patients, 24 (55.8%) were men and 19 (44.2%) were women. A Mann-Whitney test revealed no significant correlation between the intensity of MDM2 expression in men and women ($p = 0.495$). The mean Breslow index values for men and women were 1.96 ± 0.33 and 1.67 ± 0.34, respectively. An independent t-test revealed no significant difference between men and women ($p = 0.538$).

After calculating the relative frequency distribution of MDM2 expression intensity, 31 patients (72.1%) were negative, while 5 patients (11.6%), 2 patients (4.7%), and 5 patients (11.6%) were positive with weak, moderate, and strong intensity staining, respectively.

In terms of the frequency distribution of tumor thickness, 13 patients (30.2%) had tumors < 0.76 mm thick, 10 patients (23.3%) had tumors 0.76-1.5 mm thick, and 20 patients (46.5%) had tumors thicker than 1.5 mm. Spearman's correlation analysis indicated a direct relationship between tumor thickness (assessed by the Breslow index) and MDM2 expression intensity ($r = 0.425$; $p = 0.002$) (Table 1).

The level of invasion (assessed by Clark level) in the participants was classified as level I in 10 patients (23.3%), level II in 7 patients (16.3%), level III in 8 patients (18.6%), level IV in 8 patients, and level V in 10 patients (23.3%). Spearman's correlation analysis revealed a direct relationship between MDM2 expression intensity and Clark's level of invasion ($r = 0.43$; $p = 0.01$) (Table 2).

Table 1. Association between murine double minute 2 (MDM2) expression and Breslow index

Breslow index	MDM2 expression				Total
	Negative	Weakly positive	Moderately positive	Strongly positive	
Breslow <.76 count %Within MDM2	13 (41.9%)	0 (0%)	0 (0%)	0 (0%)	13 (30.2%)
Breslow .76-1.5 count %Within MDM2	7 (22.6%)	0 (0%)	0 (0%)	3 (60.0%)	10 (23.3%)
Breslow >1.5 count %Within MDM2	11 (35.5%)	5 (100.0%)	2 (100.0%)	2 (40.0%)	20 (46.5%)

Table 2. Association between murine double minute 2 (MDM2) expression and Clark's level of invasion

Clark level	MDM2 expression				Total
	Negative	Weakly positive	Moderately positive	Strongly positive	
Clark I count %Within MDM2	10 (32.3%)	0 (0%)	0 (0%)	0 (0%)	10 (23.3%)
ClarkII count %Within MDM2	6 (19.4%)	1 (20.0%)	0 (0%)	0 (0%)	7 (16.3%)
ClarkIII count %Within MDM2	5 (16.1%)	1 (20.0%)	0 (0%)	2 (40.0%)	8 (18.6%)
Clark IV count %Within MDM2	3 (9.7%)	3 (60.0%)	1 (50.0%)	1 (20.0%)	8 (18.6%)
Clark V count %Within MDM2	7 (22.6%)	0 (0%)	1 (50.0%)	2 (40.0%)	10 (23.3%)

DISCUSION

Melanoma is the cause of 74% of deaths from cutaneous cancers. Increased MDM2 expression has been observed in 50% of human cancers, including glioma, osteosarcoma, and liposarcoma. A few studies have investigated the MDM2 expression rate in malignant melanoma and its association with prognostic factors and clinical outcomes. In a study conducted by Hernberg et al., the MDM2 expression rate was, on average, low, and therefore the diagnostic significance of this factor was questioned. However, there was a significant correlation between tumor development and MDM2 overexpression.[20] In 2006, Muthusam et al. observed high-level amplification of the 12q13-15 region, a MDM2 locus site, in 3 of 53 patients with melanoma. Their analysis of mRNA and protein levels (MDM2) also revealed overexpression of the MDM2 target gene.[11] In 2000, Coupland et al. studied the prognostic significance of a number of factors, including MDM2, in 92 patients with uveal melanoma using an immunohistochemical technique. Their results revealed a significant correlation between MDM2 expression and clinical outcome (odds ratio = 2.1; p = 0.13).[18] In 2008, Firoz et al. used PCR-restriction fragment length polymorphism (RFLP) to investigate a single nucleotide polymorphism (SNP309) of the MDM2 gene. They suggested an increased risk of melanoma, especially in postmenopausal women.[6]

As these previous studies have shown, increased MDM2 expression has only been observed in a limited number of patients with malignant melanoma. In addition, immunohistochemical techniques have revealed low average MDM2 expression rates. In this study, MDM2 expression rate was also low (28%), suggesting that MDM2 is not an appropriate diagnostic factor in melanoma. In two previous studies conducted to determine immunohistochemical factors prognostic for cutaneous malignant melanoma and its clinical outcome, a strong association between MDM2 expression intensity and prognosis was observed. Similarly, direct associations between MDM2 expression intensity and tumor thickness (the most important histological prognostic factor) and Clark's level of invasion were observed in the current study.

CONCLUSIONS

In conclusion, considering the importance of immunohistochemical approaches in determining the prognosis of a large number of tumors including melanoma, this study could be clinically useful for assessing prognostic factors among patients with malignant melanoma.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55(2): 74-108.
2. Geller AC, Miller DR, Annas GD, Demierre MF, Gilchrest BA, Koh HK. Melanoma incidence and mortality among US whites, 1969-1999. JAMA 2002; 288(14): 1719-20.
3. Clark WH, Jr., Elder DE, Guerry D, Braitman LE, Trock BJ, Schultz D, et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst 1989; 81(24): 1893-904.
4. Balch CM. Cutaneous melanoma: prognosis and treatment results worldwide. Semin Surg Oncol 1992; 8(6): 400-14.
5. Buzaid AC, Ross MI, Balch CM, Soong S, McCarthy WH, Tinoco L, et al. Critical analysis of the current American Joint Committee on Cancer staging system for cutaneous melanoma and proposal of a new staging system. J Clin Oncol 1997; 15(3): 1039-51.
6. Firoz EF, Warycha M, Zakrzewski J, Pollens D, Wang G, Shapiro R, et al. Association of MDM2 SNP309, age of onset, and gender in cutaneous melanoma. Clin Cancer Res 2009; 15(7): 2573-80.
7. Iwakuma T, Lozano G. MDM2, an introduction. Mol Cancer Res 2003; 1(14): 993-1000.
8. Wallace M, Worrall E, Pettersson S, Hupp TR, Ball KL. Dual-site regulation of MDM2 E3-ubiquitin ligase activity. Mol Cell 2006; 23(2): 251-63.
9. Phan J, Li Z, Kasprzak A, Li B, Sebti S, Guida W, et al. Structure-based design of high affinity peptides inhibiting the interaction of p53 with MDM2 and MDMX. J Biol Chem 2010; 285(3): 2174-83.
10. Evans SC, Viswanathan M, Grier JD, Narayana M, El-Naggar AK, Lozano G. An alternatively spliced HDM2 product increases p53 activity by inhibiting HDM2. Oncogene 2001; 20(30): 4041-9.
11. Muthusamy V, Hobbs C, Nogueira C, Cordon-Cardo C, McKee PH, Chin L, et al. Amplification of CDK4 and MDM2 in ma-

lignant melanoma. Genes Chromosomes Cancer 2006; 45(5): 447-54.
12. Sirvent N, Coindre JM, Maire G, Hostein I, Keslair F, Guillou L, et al. Detection of MDM2-CDK4 amplification by fluorescence in situ hybridization in 200 paraffin-embedded tumor samples: utility in diagnosing adipocytic lesions and comparison with immunohistochemistry and real-time PCR. Am J Surg Pathol 2007; 31(10): 1476-89.
13. Hermanova M, Karasek P, Nenutil R, Kyr M, Tomasek J, Baltasova I, et al. Clinicopathological correlations of cyclooxygenase-2, MDM2, and p53 expressions in surgically resectable pancreatic invasive ductal adenocarcinoma. Pancreas 2009; 38(5): 565-71.
14. Kersemaekers AM, Mayer F, Molier M, van Weeren PC, Oosterhuis JW, Bokemeyer C, et al. Role of P53 and MDM2 in treatment response of human germ cell tumors. J Clin Oncol 2002; 20(6): 1551-61.
15. Li HL, Huang XP, Zhou XH, Ji TH, Wu ZQ, Wang ZQ, et al. Correlation of seven biological factors (Hsp90a, p53, MDM2, Bcl-2, Bax, Cytochrome C, and Cleaved caspase3) with clinical outcomes of ALK+ anaplastic large-cell lymphoma. Biomed Environ Sci 2011; 24(6): 630-41.
16. Rocha RM, Ignacio JA, Jordan J, Carraro DM, Lisboa B, Lopes A, et al. A clinical, pathologic, and molecular study of p53 and murine double minute 2 in penile carcinogenesis and its relation to prognosis. Hum Pathol 2012; 43(4): 481-8.
17. Da Forno PD, Saldanha GS. Molecular aspects of melanoma. Clin Lab Med 2011; 31(2): 331-43.
18. Coupland SE, Anastassiou G, Stang A, Schilling H, Anagnostopoulos I, Bornfeld N, et al. The prognostic value of cyclin D1, p53, and MDM2 protein expression in uveal melanoma. J Pathol 2000; 191(2): 120-6.
19. Foulkes WD, Stamp GW, Afzal S, Lalani N, McFarlane CP, Trowsdale J, et al. MDM2 overexpression is rare in ovarian carcinoma irrespective of TP53 mutation status. Br J Cancer 1995; 72(4): 883-8.
20. Hernberg M, Turunen JP, von BK, Muhonen T, Pyrhonen S. Prognostic value of biomarkers in malignant melanoma. Melanoma Res 1998; 8(3): 283-91.

How to cite this article: Rajabi R, Karimian P, Heidarpour M. The relationship between MDM2 expression and tumor thickness and invasion in primary cutaneous malignant melanoma. J Res Med Sci 2012; 17(5):452-5.

Source of Support: This study was funded by Isfahan University of Medical Sciences, Isfahan, Iran (research project number: 389056), **Conflict of Interest:** None declared.