Increased MDM2 expression is associated with inferior survival in mantle cell lymphoma, but not related to the MDM2 SNP309

We here show that increased expression of MDM2, a negative regulator of p53, correlates with inferior survival in a series of 43 mantle cell lymphomas. MDM2 overexpression is associated with copy number gains of the MDM2 locus in single tumors, but not with the recently reported MDM2 promoter SNP309.

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Mantle-cell lymphoma (MCL), a subtype of B-cell non-Hodgkin's lymphomas (B-NHL), is usually associated with poor clinical outcome. The genetic hallmark of MCL is the translocation t(11;14)(q13;32), resulting in overexpression of cyclin D1 and deregulation of the cell cycle. The DNA damage response pathway is also frequently affected by genetic alterations, e.g. by impairment of the ATM and p53 genes.[1] Other members of this pathway are less well studied, such as MDM2. MDM2 acts as a central negative regulator of p53, and is considered to be an oncogene.[2] MDM2 overexpression is associated with tumor progression and predicts decreased survival in solid tumors and hematological malignancies.[3,4] In MCL, a subset of cases shows increased levels of MDM2 expression, but only single cases harbor genomic gains or amplifications of the MDM2 locus.[5] Thus, the increased MDM2 expression often remains unexplained. Recently, a single nucleotide polymorphism (SNP) in the MDM2 promotor (T309G) was found to increase the affinity of the transcription factor Sp1, leading to enhanced expression of MDM2.[6] Several reports have linked the presence of this SNP to an increased risk and/or earlier onset of malignant tumors.[7]

In the present study we investigated the MDM2 gene expression levels of 43 MCL samples in relationship to clinical outcome, genomic status of MDM2 and presence of MDM2 SNP309. Frozen tumor samples were obtained from the Institute of Pathology, University of Würzburg and the Hospital Clinic, University of Barcelona. Thirty-two cases showed typical and 11 blastoid morphology. The proliferation fraction was evaluated by Ki-67 staining as previously described.[8] RNA and DNA were isolated according to standard protocols. MDM2 gene expression levels were determined by Taqman™ Real-time quantitative RT-PCR with pre-developed assays (Applied Biosystems, MDM2: Hs_00234753_m1, B2M: Hs_00187842_m1) and calculated with the 2-ΔΔCt method using B2M as endogenous control. MDM2 genomic status was assessed using a quantitative PCR approach as previously described.[5] The MDM2 SNP309 was examined by direct sequencing, as described6 and validated by subcloning into the pCR2.1TA cloning vector (Invitrogen) and sequencing. The mutational status of p53 was studied by sequencing (36 cases) as reported.[9] The association between MDM2 gene expression and survival was determined with the Cox hazard regression model. p values for the associations between continuous and qualitative variables were calculated using an analysis of variance test. The χ^2 test was applied to analyze categorical data. p values <0.05 were considered significant.

The median survival of MCL patients in our series was 48 months. MDM2 gene expression was highly variable, showing a 14-fold range between the cases with highest and lowest expression (Figure 1A). Importantly, increased expression was associated with poor prognosis in this series (p=0.006, Figure 1B). On the genomic level, three MCL cases showed evidence of an allelic gain of the MDM2 locus, correlating with increased MDM2 expression (p=0.005). However, increased MDM2 expression was present in many additional MCL tumors which did not harbor chromosomal gains in the MDM2 locus (Figure 1A). Since MDM2 gene expression levels are influenced by the MDM2 SNP3096, we studied its potential impact on MDM2 expression in our series of MCL. The observed MDM2 SNP309 frequencies correlated well with the frequencies reported in a healthy population[6] (Table 1). The presence of the SNP309 was not related to MDM2 mRNA expression (Figure 1C; p=0.74), age at diagnosis, morphological subtype and Ki-67 index. Likewise, there was no correlation between the presence of the SNP309 and survival. Interestingly, the G allele of SNP309 was observed in 9 out of ten female patients (Table 1; p=0.029). Several studies suggest that the MDM2 SNP309 may accelerate tumor formation in a

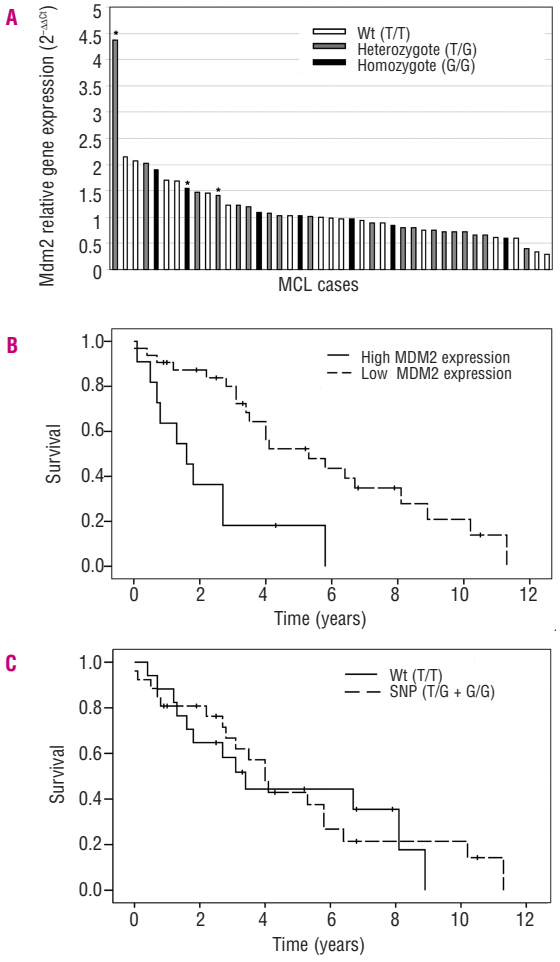

Figure 1. A. MDM2 gene expression levels in 43 MCL cases measured by real-time quantitative RT-PCR. Expression levels were calculated with the relative 2 -ΔΔCt method, using B2M as an endogenous control. Different bar colors depict the status of the SNP309 in the MDM2 promoter. Samples with a genomic copy number gain of the MDM2 locus are marked with an asterisk. Kaplan-Meier plots visualizing survival groups according to **B.** MDM2 mRNA expression (cutoff >75% percentile), and **C.** SNP309 status.

Table 1. Summary of the MDM2 SNP309 status in 43 MCL cases and association with age at diagnosis, sex, histology, proliferation fraction (Ki-67) and p53 status (wt: wild-type; mut: p53 mutation).

SNP status	Frequency (cases, %)	Age at diagnosis (years)	Sex		Histology		Ki67 (%)	P53	
			Male	Female	Common	Blastoid		Wt	mut
Wildtype (T/T)	17 (39.5%)	64.1	16	1	14	3	35	12	2
Heterozygot (T/G)	19 (44.2%)	63.2	12	7	14	5	35	10	5
Homozygot (G/G)	7 (16.4%)	63.7	5	2	4	3	32	6	1
G allele (T/G+G/G)	26 (60.5%)	63.3	17	9	18	8	34	16	6
Total			33	10	32	11		28	8

gender and hormone specific way in women.[7] In MCL, the presence of the G allele in women showed no association with younger age of tumor onset or survival when compared to men. 36 MCL cases were further analyzed for the p53 status, and 8 of them showed p53 mutations. As previously described10, p53 mutations were found to be associated with poor prognosis in our series (p=0.036). However, no correlation between the presence of the MDM2 SNP309 or MDM2 mRNA expression and p53 status was observed.

In conclusion, we provide evidence that besides p53 alterations, increased gene expression levels of MDM2 are directly correlated with inferior survival in MCL patients, underlining the importance of the DNA damage response pathway for the pathogenesis of MCL. In single MCL tumors, genomic gains of the MDM2 locus probably account for elevated MDM2 expression levels, while the presence of the SNP309 has no impact.

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Key words: mantle cell lymphoma, MDM2 expression, survival, SNP309

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References

1. Fernandez V, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. J Clin Oncol 2005; 23:6364-9.
2. Brooks CL, Gu W. p53 ubiquitination: Mdm2 and beyond. Mol Cell 2006; 21:307-15.
3. Turbin DA, Cheang MC, Bajdik CD, Gelmon KA, Yorida E, De Luca A, et al. MDM2 protein expression is a negative prognostic marker in breast carcinoma. Mod Pathol 2006; 19:69-74.
4. Moller MB, Nielsen O, Pedersen NT. Oncoprotein MDM2 overexpression is associated with poor prognosis in distinct non-Hodgkin's lymphoma entities. Mod Pathol 1999; 12:1010-6.
5. Hernandez L, Bea S, Pinyol M, Ott G, Katzenberger T, Rosenwald A, et al. CDK4 and MDM2 gene alterations mainly occur in highly proliferative and aggressive mantle cell lymphomas with wild-type INK4a/ARF locus. Cancer Res 2005; 65:2199-206.
6. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 2004; 119:591-602.
7. Bond GL, Hirshfield KM, Kirchhoff T, Alexe G, Bond EE, Robins H, et al. MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res 2006;66:5104-10.
8. Katzenberger T, Petzoldt C, Holler S, Mader U, Kalla J, Adam P, et al. The Ki67 proliferation index is a quantitative indicator of clinical risk in mantle cell lymphoma. Blood 2006;107:3407.
9. Pinyol M, Hernandez L, Martinez A, Cobo F, Hernandez S, Bea S, et al. INK4a/ARF locus alterations in human non-Hodgkin's lymphomas mainly occur in tumors with wild-type p53 gene. Am J Pathol 2000;156:1987-96.
10. Greiner TC, Moynihan MJ, Chan WC, Lytle DM, Pedersen A, Anderson JR, et al. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. Blood 1996;87:4302-10.