Mdm2-SNP309 polymorphism in prostate cancer: no evidence for association with increased risk or histopathological tumour characteristics

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The search for inherited cancer susceptibility factors is a major focus of epidemiologic cancer studies. Analyses of single-nucleotide polymorphisms (SNP) in a variety of genes revealed a correlation between a specific allele variant and cancer predisposition. Human mouse double-minute 2 protein (Mdm2) is a cellular E3 ligase capable of ubiquitination and degradation of p53. Therefore, Mdm2 is a crucial factor of cell cycle control and cell survival. The Mdm2 promoter SNP309 was shown to increase Mdm2 expression and can, thereby, inhibit the p53 pathway. This SNP was found to be associated with increased risk and early onset of various malignancies. For prostate cancer no studies are reported to date. In a case–control study we determined the distribution of the Mdm2 SNP309 in 145 male subjects with prostate cancer and in 124 male controls without any malignancy using RFLP analysis. Cases and controls showed a similar distribution of the SNP (P = 0.299). Genotype distribution showed neither an association with histopathological characteristics of the tumours nor with prognosis. Age at disease onset was also not modified by the SNP. This first study of the Mdm2 SNP309 in prostate cancer patients suggests no correlation between a certain allelic variant and an increased cancer risk.

Keywords: prostate cancer; Mdm2 SNP309; RFLP; p53 pathway

Genomic integrity and the ability of accurate repair of cellular damage are crucial prerequisites for controlled proliferation and differentiation. These processes are highly regulated and disruption of components of this network favours malignant transformation.

The p53 protein is one of the most important coordinators of cellular response to genotoxic stress (Levine, 1997). The tumour suppression functions of p53 are widespread and mediated by various mechanisms. p53 is tightly regulated and the exact mechanism of p53 activation are still not fully understood (Brooks and Gu, 2006). Several studies have shown that the major mechanism of p53 control is its degradation by the ubiquitin-proteasome pathway (Brooks and Gu, 2006). Human mouse double-minute 2 protein (Mdm2) (gene locus on chromosome 12q14.3-q15) is a cellular E3 ligase capable of ubiquitinating and degrading of p53 (Haupt et al, 1997). As a key negative regulator of p53, overexpression of Mdm2 is associated with accelerated tumour progression and lack of response to therapy in various human malignancies (Bond et al, 2004). Besides gene amplification, a naturally occurring single-nucleotide polymorphism (SNP) in the promoter of the Mdm2 gene (SNP309, T>G) was found that increases mRNA and protein levels (Bond et al, 2004). Several studies have analysed the impact of the Mdm2 SNP309 on cancer risk in sporadic and hereditary malignancies and a recently published meta-analysis revealed that there is only little effect on the risk of common cancers from this SNP alone. Nevertheless, a modification of the onset of tumour formation and the individual prognosis might be influenced by this sequence variation (Wilkening et al, 2007). Interestingly, Bond and co-workers found an acceleration of tumour formation in a gender-specific and hormone-dependent manner by this polymorphism (Bond et al, 2006).

Prostate cancer (PCa) poses one of the major health care problems today. PCa is responsible for approximately 11% of all male cancers in Europe, and about 9% of all cancer deaths in men are caused by this malignancy within the European Union, the course of this malignancy is unpredictable and therapy not warranted in all patients (Heidenreich et al, 2008). A variety of treatment options ranging from watchful waiting to radical surgery underlines the necessity for the identification of reliable factors permitting individual risk assessment.

Major progress in discovery of SNPs has lead to a rapid increase of genetic association studies in various malignancies including PCa. Despite promising results no specific PCa-related genes have been established yet. However, the involvement of variants in androgen pathway genes in PCa aetiology could recently be confirmed reproducibly (Lindström et al, 2006).

Alterations in the p53 pathway are already described in PCa including Mdm2 protein overexpression in about 30–45% of the
analysed tumours. This overexpression was associated with advanced tumour stage, higher tumour volume and increased cell proliferation (Osman et al., 1999; Leite et al., 2001; Khor et al., 2005). Although these data indicate a potential role of Mdm2 over-expression in PCa, there have been no studies published investigating the distribution of the functional Mdm2 SNP309 in PCa patients to date. Therefore, the aim of the presented study was the analysis of the allele frequencies of the Mdm2 SNP309 in patients with PCa compared to a healthy control group to assess a possible influence of this promoter alteration on cancer risk, histopathological tumour characteristics and prognosis.

MATERIALS AND METHODS

Samples

Overall, 145 patients with PCa were included in our study. All PCa patients underwent a radical prostatectomy. Formalin-fixed and paraffin-embedded tissue samples from these patients were available from prior transurethral resection (TUR-P, n = 31) or from the prostatectomy specimens (n = 114). For comparison 124 samples from a male control group of patients without any malignancy acquired at the Department of Urology, University of Regensburg was investigated.

All tumours were diagnosed according to the 2004 WHO classification of prostate tumours (Epstein et al., 2004) and staged according the TNM system (Sobin and Wittekind, 2002). Characteristics of the study participants are shown in Table 1. Detailed clinical information was available from 65 PCa patients. Clinical follow-up (mean 71 months and range 6 – 144 months) revealed that 20 tumours showed a recurrence within 3 years (defined as detectable serum prostate specific antigen (PSA) following radical prostatectomy (>0.2 ng ml⁻¹)), whereas 45 tumours did not recur within at least 5 years. Prior IRB approval was obtained for the study.

Tissue microdissection and DNA isolation

DNA was extracted from normal prostate tissue or peripheral blood using the High Pure PCR Template Preparation Kit (Roche GmbH, Mannheim, Germany) according to the manufacturer’s instructions.

Mdm2 SNP309 analysis

Single-nucleotide polymorphism analysis was carried out by restriction fragment length polymorphism analysis (RFLP) of the promoter region which contains an Msp I site (5’-CGCGCGTCAGGGTAAG-3’; antisense: 5’-CTGAGTCACCGGCACTG-3’). Obtained from Metabion (Martinsried, Germany) in a total volume of 25 µl containing approximately 100 ng DNA, 0.2 mM dNTP (Roche Diagnostics), 0.18 µM primers and 0.0025 U GoTaq (Promega, Mannheim, Germany). The thermal cycling conditions were as follows: initial denaturation for 3 min at 95 °C, 35 cycles of denaturation at 95 °C for 1 min, annealing at 61 °C for 1 min, elongation at 72 °C for 1 min and final primer extension at 72 °C for 10 min.

PCR products were incubated overnight with 5 U Msp A11 (New England Biolabs, Frankfurt/Main, Germany) and 100 µg ml⁻¹ BSA at 37 °C in a total volume of 30 µl to ensure complete digestion. Restriction fragments were separated by capillary electrophoresis using an ABI PRISM 310 genetic analyser (Applied Biosystems, Foster City, CA) and analysed with the GeneScan Analysis Software. Representative examples of genotyping are shown in Figure 1. Ten randomly selected cases were also sequenced to verify the RFLP results. In all cases identical results were obtained (data not shown).

Statistical analysis

To test if the genotype distribution followed Hardy–Weinberg equilibrium, the public software at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl was used. χ² statistics (two-sided Fisher’s exact test) were used to evaluate case-control differences in the distribution of genotypes and to analyse associations between genotypes and clinical or histopathological characteristics. P < 0.05 was interpreted as statistically significant.

RESULTS

All analysed samples gave interpretable results. The genotype distribution in our cohorts followed the Hardy–Weinberg equilibrium in cases (P = 1.000) and controls (P = 0.578). Genotype distribution between cases and controls did not differ significantly. Although there was a higher frequency of T/T genotypes in prostate cancer patients this difference did not reach statistical significance (P = 0.299, Table 2, Figure 2). There was also

### Table 1  Characteristics of study participants

|                  | Cases  | Controls |
|------------------|--------|----------|
| Number:          | n = 145| n = 124  |
| Age:             |        |          |
| Median:          | 65     | 69       |
| Mean:            | 64.2 ± 6.0| 68.5 ± 9.9|
| Stage:           |        |          |
| pT1-3a           | n = 105|          |
| pT3b-3c          | n = 28 |          |
| No data available| n = 12 |          |
| Gleason score:   |        |          |
| Median:          | 7      | 3        |
|                    | Range: 3–10 | 40–88 |
| Gleason sum:     |        |          |
| 3–4              | n = 3  |          |
| 5–7              | n = 103 |        |
| 8–10             | n = 27 |          |
| No data available| n = 12 |          |
| Recurrence:      |        |          |
| Recurrence within 3 years | n = 20 |        |
| No recurrence within 5 years | n = 45 |          |
| No data available| n = 80 |          |
no association between overall disease risk and presence of the polymorphic promoter variant (T/T vs T/G + G/G; \( P = 0.131 \), Table 2). In addition, we also did not observe any significant correlation of genotypes and disease recurrence or Gleason score (Table 3). As the Mdm2 SNP309 was previously shown to be related with disease onset at younger age also in sporadic tumours (eg, bladder cancer (Sanchez-Carbayo et al, 2007)) we tested our cases towards this hypothesis. Within our cohort there was no significant association between genotypes and early disease onset (\( \leq 60 \) years vs \( > 60 \) years, Table 3).

### DISCUSSION

The Mdm2 SNP309 is a plausible cancer predisposing allele due to the crucial role of Mdm2 in the cellular p53 pathway. The T to G variant was shown to result in increased Mdm2 synthesis and was found to be correlated with the risk of cancer or an early onset of tumour formation at various organ sites (Hu et al, 2007). In this study, we genotyped 145 cases of PCa and 124 male controls for this polymorphism. In our cohort, we did not find any association of the SNP309 with tumour risk, age at tumour onset, histopathological characteristics of the tumours or prognosis.

Overexpression of Mdm2 and its clinical consequences were already described in PCa (Osman et al, 1999; Leite et al, 2001; Khor et al, 2005) but the molecular basis of this upregulation is still unclear. As our study revealed no significant changes in the allelic distribution between PCa patients and men without any malignancy, it is unlikely that the Mdm2 SNP309 plays an important role for increased Mdm2 expression in prostate tumours. Moreover, chromosomal deletions at 12q have not been described in PCa to date, making a preferential loss of the Mdm2 T-allele resulting in a Mdm2 overexpression during PCa development unlikely (Dumur et al, 2003).

Mdm2 overexpression could be linked to gene amplification in several malignancies (eg, malignant melanoma, non-small cell lung cancer and lipomatous tumours (Dworakowska et al, 2004; Nilsson et al, 2004; Muthusamy et al, 2006) but in PCa a specific Mdm2 gene amplification could not be demonstrated by Southern blot analysis so far (Ittmann et al, 1994). Interestingly, frequent gains of chromosome 12q including the regions 12q13-q14 that are in close proximity to the Mdm2 gene locus have been reported in various studies using comparative genomic hybridisation (Sattler et al, 1999; Zitzelsberger et al, 2001) but to date no gene copy number...

### Table 2

| Genotype | Cases (%) | Controls (%) | Comparison |
|----------|-----------|--------------|------------|
| TT       | 61 (42.1) | 41 (33.1)    | \( P = 0.299 \) |
| GT       | 66 (45.5) | 64 (51.6)    |            |
| GG       | 18 (12.4) | 19 (15.3)    | \( P = 0.131 \) OR: 0.680 95% CI: 0.413 – 1.120 |
| TG + GG  | 84 (57.9) | 83 (66.9)    |            |

### Figure 1

Representative examples for RFLP analyses. (A) T/G genotype resulting in an undigested 157 bp product and a digested 51 bp product. The second fragment from the Msp A1I digest (106 bp) is not visible due to 5’-FAM-labeling of the PCR product. (B) T/T genotype showing only the 157 bp PCR product after Msp A1I digest. (C) G/G genotype displays only the 51 bp after digest of the PCR product.

### Figure 2

Graphical illustration of the genotype distribution in cases and controls.
analysis specific for Mdm2 using highly sensitive, fluorescence-based methods has been performed.

The G-variant of the Mdm2 promoter polymorphism increases the affinity of the transcription factors for example, Sp1. As long as Mdm2 gene amplification is not analysed in detail in PCA, overexpression of Sp1 might be speculated as an activator of increased Mdm2 synthesis. Numerous studies have documented that Sp1 activity and/or Sp1 expression levels are elevated in various human cancers and are associated with prognosis (Safe and Abdelrahim, 2005; Deniaud et al, 2006). In PCs elevated Sp1 levels have not been described so far but an increased expression of Sp2 in PCa cells was found recently (Phan et al, 2004) indicating a possible role of deregulation of Sp/KLF family member expression in PCa.

Mdm2 is still discussed as a promising target for a therapeutical approach (Vassilev, 2007). The identification of the first selective and potent inhibitors of the p53-Mdm2 interaction emphasised the usage of small-molecule inhibitors as viable alternative to chemotherapy for selective p53 activation in tumours with wild-type p53. In PCa p53 alterations are a rare event in primary tumours and associated with more aggressive disease, metastasis and transition from androgen-dependent to androgen-indepen-dent growth (Dong, 2006). Therefore, a subset of prostate tumours with Mdm2 overexpression might evolve as a suitable target for the application of selective Mdm2 inhibitors in the future.

In conclusion, our case–control study suggests that the Mdm2 promoter polymorphism SNP309 has no influence on PCA risk or prognosis, and relevant inherited alterations in PCA-related genes remain to be uncovered.

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Conflict of interest

The authors state no conflict of interest.

REFERENCES

Bond GL, Hirshfield KM, Kinchhoff T, Alexe G, Bond EE, Robins H, Bartel F, Taubert H, Wuerl P, Hait W, Toppmeyer D, Offit K, Levine AJ (2006) MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res 66: 5104 – 5110

Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591 – 602

Brooks CL, Gu W (2006) p53 ubiquitination: Mdm2 and beyond. Mol Cell 21: 307 – 315

Deniaud E, Baquet J, Mathieu AL, Pages G, Marvel J, Leverrier Y (2006) Overexpression of Sp1 transcription factor induces apoptosis. Oncogene 25: 7096 – 7105

Dong JT (2006) Prevalent mutations in prostate cancer. J Cell Biochem 97: 433 – 447

Dumur CI, Dechakhum C, Ware JE, Cofield SS, Best AM, Wilkinson DS, Garrett CT, Ferreira-Gonzalez A (2003) Genome-wide detection of LOH in prostate cancer using human SNP microarray technology. Genomics 81: 260 – 269

Garrett CT, Ferreira-Gonzalez A (2003) Genome-wide detection of LOH from 21 case-control studies. Cancer Epidemiol Biomarkers Prev 12: 16: 68 – 80

Heidenreich A, Aus G, Bolla M, Joniau S, Allsbrook WC, Bastacky S, Boccon-Gibod L, De Marzo AM, Egevad L, Furusato M, Hamper UM, Helpap B, Humphrey PA, Iczkowski KA, Lopez-Beltran A, Montironi R, Rubin MA, Sakr WA, Stamey TA, Parkin DM (2004) Tumours of the prostate. In Pathology and Genetics. Tumours of the Urinary System and Male Genital Organs, World Health Organization, classification of tumours. pp 159 – 216. IARC, Lyon

Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387: 296 – 299

Humphrey PA, Iczkowski KA, Lopez-Beltran A, Montironi R, Rubin MA, Sakr WA, Stamey TA, Parkin DM (2004) Tumours of the prostate. In Pathology and Genetics. Tumours of the Urinary System and Male Genital Organs, World Health Organization, classification of tumours. pp 159 – 216. IARC, Lyon

Heidenreich A, Aus G, Bolla M, Joniau S, Allsbrook WC, Bastacky S, Boccon-Gibod L, De Marzo AM, Egevad L, Furusato M, Hamper UM, Helpap B, Humphrey PA, Iczkowski KA, Lopez-Beltran A, Montironi R, Rubin MA, Sakr WA, Stamey TA, Parkin DM (2004) Tumours of the prostate. In Pathology and Genetics. Tumours of the Urinary System and Male Genital Organs, World Health Organization, classification of tumours. pp 159 – 216. IARC, Lyon

Hyten DL, Deininger PL, Sannerstedt M, Wilson RM, Meuwese MA, Friend SH, Page GP (2000) Large-scale analysis of gene expression using expression mapping by suppression subtractive hybridization. Genome Res 10: 1715 – 1722

Lozano G, Levine AJ (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591 – 602

Nature 387: 296 – 299

Overexpression of Sp1 transcription factor induces apoptosis. Oncogene 25: 7096 – 7105

Dong JT (2006) Prevalent mutations in prostate cancer. J Cell Biochem 97: 433 – 447

Dumur CI, Dechakhum C, Ware JE, Cofield SS, Best AM, Wilkinson DS, Garrett CT, Ferreira-Gonzalez A (2003) Genome-wide detection of LOH in prostate cancer using human SNP microarray technology. Genomics 81: 260 – 269

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Table 3 Distribution of allelic variants between relevant tumour/patient characteristics

| Genotype | Cases with recurrence (%) | Cases without recurrence (%) | Comparison |
|----------|--------------------------|-----------------------------|------------|
| TT       | 8 (40)                   | 13 (28.9)                   | P = 0.688  |
| GT       | 10 (50)                  | 27 (60)                     | P = 0.402  |
| GG       | 2 (10)                   | 5 (11.1)                    | OR: 1.641  |
| TT       | 8 (40)                   | 13 (28.9)                   | 95% CI: 0.361 – 1.514 |
| TG+GG    | 12 (60)                  | 32 (71.1)                   |            |

Gleason sum < 7 (%)  Gleason sum ≥ 7 (%)  
TT 19 (35.9) 34 (43)  TT 19 (35.9) 34 (43)
GT 27 (50.9) 36 (45.6)  GT 27 (50.9) 36 (45.6)
GG 7 (13.2) 9 (11.4)  GG 7 (13.2) 9 (11.4)
TT 19 (36) 34 (43)  TT 19 (36) 34 (43)
TG+GG 34 (64) 45 (57)  TG+GG 34 (64) 45 (57)

Age < 60 (%)  Age > 60 (%)  
TT 11 (35.5) 40 (42.1)  TT 11 (35.5) 40 (42.1)
GT 15 (48.4) 36 (45.6)  GT 15 (48.4) 36 (45.6)
GG 5 (16.1) 9 (28.9)  GG 5 (16.1) 9 (28.9)
TT 11 (35.5) 40 (42.1)  TT 11 (35.5) 40 (42.1)
TG+GG 20 (64.5) 55 (57.9)  TG+GG 20 (64.5) 55 (57.9)

Cl = confidence interval; OR = odds ratio.
Ittmann M, Wieczorek R, Heller P, Dave A, Provet J, Krolewski J (1994) Alterations in the p53 and MDM-2 gene are infrequent in clinically localized, stage B prostate adenocarcinomas. Am J Pathol 145: 287 – 293
Khor LY, Desilvio M, Al-Saleem T, Hammond ME, Grignon DJ, Sause W, Pilepich M, Okunieff P, Sandler H, Pollack A (2005) MDM2 as a predictor of prostate carcinoma outcome: an analysis of radiation therapy oncology group protocol8610. Cancer 104: 962 – 967
Leite KR, Franco MF, Srougi M, Nesrallah IJ, Nesrallah A, Bevilacqua RG, Darini E, Carvalho CM, Meirelles MI, Santana I, Camara-Lopes LH (2001) Abnormal expression of MDM2 in prostate carcinoma. Mod Pathol 14: 428 – 436
Levine AJ (1997) p53, the cellular gatekeeper for growth and division. Cell 88: 323 – 331
Lindström S, Zheng SL, Wiklund F, Jonsson BA, Adami HO, Bäler KA, Brookes AJ, Sun J, Chang BL, Liu W, Li G, Isaacs WB, Adolfsson J, Grönlund H, Xu J (2006) Systematic replication study of reported genetic associations in prostate cancer: strong support for genetic variation in the androgen pathway. Prostate 66: 1729 – 1743
Muthusamy V, Hobbs C, Nogueira C, Cordon-Cardo C, McKee PH, Chin L, Rosenberg MW (2006) Amplification of CDK4 and MDM2 in malignant melanoma. Genes Chromosomes Cancer 45: 447 – 454
Nilsson M, Meza-Zepeda LA, Mertens F, Forus A, Myklebost O, Mandahl N (2004) Amplification of chromosome 1 sequences in lipomatous tumors and other sarcomas. Int J Cancer 109: 363 – 369
Osman I, Drobnjak M, Fazzari M, Ferrara J, Scher HI, Cordon-Cardo C (1999) Inactivation of the p53 pathway in prostate cancer: impact on tumor progression. Clin Cancer Res 5: 2082 – 2088
Phan D, Cheng CJ, Galfone M, Vakar-Lopez F, Tunstead J, Thompson NE, Burgess RR, Najjar SM, Yu-Lee LY, Lin SHL (2004) Identification of Sp2 as a transcriptional repressor of carcinoembryonic antigen-related cell adhesion molecule 1 in tumorigenesis. Cancer Res 64: 3072 – 3078
Safe S, Abdelrahim M (2005) Sp transcription factor family and its role in cancer. Eur J Cancer 41: 2438 – 2448
Sanchez-Carbayo M, Socci ND, Kirchoff T, Erill N, Offit K, Bochner BH, Cordon-Cardo C (2007) A polymorphism in HDM2 (SNP309) associated with early onset in superficial tumors, TP53 mutations, and poor outcome in invasive bladder cancer. Clin Cancer Res 13: 3215 – 3220
Sattler HP, Rohde V, Bönkoff H, Zwergel T, Wullich B (1999) Comparative genomic hybridization reveals DNA copy number gains to frequently occur in human prostate cancer. Prostate 39: 79 – 86
Sobin LH, Wittekind C (2002) TNM Classification of Malignant Tumors. Wiley & Sons, New York
Sotomaa K, Liyanarachchi S, Mecklin JP, Järvinen H, Aaltonen LA, Peltimäki P, de la Chapelle A (2005) p53 codon 72 and Mdm2 SNP309 polymorphisms and age of colorectal cancer onset in Lynch syndrome. Clin Cancer Res 11: 6840 – 6844
Vassilev LT (2007) MDM2 inhibitors for cancer therapy. Trends Mol Med 13: 23 – 31
Wilkening S, Bermejo JL, Hemminki K (2007) MDM2 SNP 309 and cancer risk: a combined analysis. Carcinogenesis 28: 2262 – 2267
Zitzelsberger H, Engert D, Walch A, Kulka U, Aubele M, Höfler H, Bauchinger M, Werner M (2001) Chromosomal changes during development and progression of prostate adenocarcinomas. Br J Cancer 84: 202 – 208