Correlation of DNA Mismatch Repair Protein hMSH2 Immunohistochemistry with p53 and Apoptosis in Cervical Carcinoma

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Abstract. Background: Mutations in genes of the DNA mismatch repair system (MMR) are linked to hereditary non-polyposis colorectal cancer and also play a role in sporadic cancer. Besides its repair function, the MMR is the linkage of DNA mismatch recognition to the cell cycle control. Materials and Methods: The correlation between the immunoreactivity of the MMR protein hMSH2 and p53, apoptosis, clinical prognosis factors and the survival rate in 102 samples of cervical carcinoma was determined. Results: hMSH2 immunoreactivity was correlated with p53 and weakly correlated with apoptosis. hMSH2 immunoreactivity could not be correlated to any tumour markers, while apoptosis correlated significantly with T stage, FIGO classification and the relative risk of death from cervical cancer. Conclusion: In cervical cancer, the processes of DNA mismatch repair, cell cycle control and apoptosis seemingly act in concert. Decreased expression of the hMSH2 mismatch repair protein might lead to a failure in the induction of apoptosis.

An association between the DNA mismatch repair (MMR) protein, Mut-S-homologon-2 (hMSH2) and cancer was first described in 1993 in hereditary non-polyposis colorectal cancer (HNPCC) (1-3). The loss of MMR function in HNPCC results in a high frequency of microsatellite instabilities (MSI), the impairment of chromosomal integrity and promotes tumorigenesis. As part of the MMR complex, hMSH2 binds to DNA base-to-base mismatches as a heterodimer with hMSH6 (MutSα), or to insertion- and deletion-loop mismatches as a heterodimer with hMSH3 (MutSβ) (4). The MutLα heterodimer comprising the Mut-L-homologon-1 (hMLH1) and postmeiotic-segregation-increased-2 (PMS2) binds to the MutS heterodimer. The replication processivity factor proliferating-cell-nuclear-antigen (PCNA) links the MMR complex to an enzyme complex which exchanges the mispaired part of the mutated strand (5, 6).

Defects or altered expression of MMR-proteins were not only found in HNPCC, but also in a number of sporadic cancers, where they were not necessarily linked to the appearance of MSI (7-10). In gynaecological cancer, hMSH2 alterations were found, e.g. in endometrial cancer (11, 12), ovarian cancer (13, 14), malignancies of the uterine corpus (15) and breast cancer (16-18). Only a few studies focused on hMSH2 and cervical cancer. Two describe an intensified expression of hMSH2 in malignant tissues compared with non-neoplastic samples (19, 20). An association between cancer and MMR is not necessarily linked to the loss of immunoreactivity for one of the MMR proteins since a mutation of hMSH2, with the subsequent loss of MMR-function, was identified in breast cancer to be still immunoreactive (21).

On chromosomal damage the MMR proteins can activate proteins of cell cycle checkpoints and apoptosis by an as yet unclear mechanism (22). An interaction of PMS2 with p73, an associate of the tumour suppressor p53, is necessary for the induction of apoptosis after cisplatin administration (23). Point mutations in hMSH2 resulted in partially functional proteins that made it possible to distinguish between the mismatch repair function of hMSH2 and its ability to induce cell death after cisplatin exposure (24, 25). The tumour suppressor protein p53 inhibits tumorigenesis by cell cycle arrest and induction of apoptosis. Mutations in this central gatekeeper of the cell cycle are involved in a high percentage of cancer. Scherer et al. (26) identified a response element for p53 in the promoter region of the hMSH2 gene and Warnick et al. showed that p53 actively regulates the hMSH2 expression in ovarian cancer cells (27).
The relation between hMSH2 and p53 has been investigated in various studies with diverse outcomes in different types of cancer. Rass et al. (28) found increased immunoreactive scores in malignant melanoma for both hMSH2 and p53, whereas Spagnoletti et al. found hMSH2-positive tumour cells tended to be negative for the expression of p53 in breast cancer (10).

In this study the relationship between the expression of the MMR protein hMSH2, p53 and apoptosis was investigated in samples of cervical carcinoma. Further, the correlation of these results with prognostic factors in cervical carcinoma, such as steroid receptor status, TNM stage, FIGO classification and tumour grading, as well as with the survival rate, was determined.

Materials and Methods

Tissue specimens. Tumour tissue from 102 patients with cervical carcinoma was collected. The investigations on human material were approved by the ethics-committee of the University of Homburg/Saar. Each patient consented to participate in this study. Steroid receptor expression and the tumour staging (T stage=tumour size and character; N stage=modal status; M stage=distant metastases), histopathological staging and FIGO grading were taken from the reports of the pathologists.

Immunohistochemistry. Freshly excised tissue samples from cervical carcinoma were fixed in 4% phosphate-buffered paraformaldehyde for 24 h at 4°C and embedded in paraffin. Serial sections of 6-µm thickness were cut on a rotation microtome and mounted on microscope slides previously silanised and treated with 0.5% ovalbumin. After drying, slides were deparaffinized in xylol and rehydrated in descending concentrations of alcohol, submerged in 3% hydrogen peroxide to prevent endogenous peroxidase activity and demasked in a microwave oven at 500 W for 10 min in 0.1 M citrate-buffer. Slides were then washed with Tris-buffer. Non-specific protein binding was blocked with normal rabbit sera (DAKO, Hamburg, Germany; 1:50 in Tris-buffer) for 15 min.

As primary antibody for hMSH2, the monoclonal mouse antibody clone FE-11 was used at a dilution of 1:30 (Calbiochem, Schwalbach, Germany), for p53 the monoclonal mouse antibody clone DO-7, at a 1:30 dilution and for Ki-67 the polyclonal rabbit antibody A-047 was used at a 1:50 dilution (both DAKO, Hamburg, Germany), for p53 the monoclonal mouse antibody clone FE-11 was used at a dilution of 1:20 (Calbiochem, Schwalbach, Germany), for p53 the monoclonal mouse antibody clone DO-7, at a 1:30 dilution and for Ki-67 the polyclonal rabbit antibody A-047 was used at a 1:50 dilution (both DAKO, Hamburg, Germany). The primary antibodies were incubated on the tumour sections overnight at 4°C.

Immunodetection was performed using the ABC method (DAKO, Hamburg, Germany). The secondary biotinylated antibody was incubated for 15 min at 37°C followed by the streptavidin-biotin-complex for 15 min at 37°C and an amplification reaction with streptavidin-peroxidase and biotinyl-tyramine for another 15 min at 37°C. Three wash steps were performed after all incubations. Bound peroxidase activity was visualised with 3,3’-diaminobenzidine (DAB) (DAKO, Hamburg, Germany).

Immunoreactive detection of apoptosis after TdT-mediated-dUTP-nick-end-labelling-(TUNEL) assay. For the detection of apoptotic cells in tissue sections the In-Situ-Cell-Death-Detection-Kit, AP was used (Roche, Mannheim, Germany). The slides were deparaffinized and rehydrated. Proteinaise-K digestion was performed for 25 min at room temperature. The TUNEL reaction was undertaken at 37°C for 1 h under a cover glass.

For the detection of fluorescein-dUTP-labelled cells, an alkaline phosphatase-linked antifluorescein antibody was used. The immunoreaction was visualised with the New-Fuchsin-Substrate-Kit (DAKO, Hamburg, Germany).

Interpretation of immunoreactivity. The immunoreactive score (IRS) described by Remmele and Stegner (29) was used for the interpretation of the immunoreactivity in tumour sections. The IRS is the product of the staining intensity (SI: negative=0; weak=1; moderate=2; strong=3) and the percentage score of immunopositive cells (PP: 0=no positive cells; 1=1-10%; 2=11-50%; 3=51-80%; 4=>80% positive cells). Immunoreactivity for Ki-67 was evaluated and compared with other immuno-reactivities with a scoring after Friedrich et al. (15) that creates a ranking of the percentages of immunopositive cells (PP) (rank 0=0%; 1=1-10%; 2=11-25%; 3=26-50%; 4=>50% positive cells). Statistical analysis of correlation.

Microscopy and photography. The stained sections were viewed under a Leica DM4000 microscope and photographed using an SIS Colorview 12 digital camera.

Statistical analysis of correlation. The statistical correlations among hMSH2, p53, Ki-67 and apoptosis immunoreactivity, as well as the correlations with pathological data, were analysed using Spearman’s rank correlation co-efficient in SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at p<0.05.

Results

We examined cervical carcinoma samples from 102 patients using immunohistochemistry for hMSH2, p53, Ki-67 and TUNEL-reactivity for apoptosis detection. Examples of sections after immunostaining are shown in Figure 1.

Immunoreactivity for hMSH2, p53, Ki-67 proteins and for apoptosis. The results from immunodetection with the antibodies for hMSH, p53 apoptosis and Ki-67 are presented in Table I. The IRS state the mean of immunoreactivity scores of all samples. For Ki-67 only the PP-score representing the number of positive cells is shown. All antibodies reacted positively with the majority of the samples with the highest IRS for hMSH2.
Correlations of the immunoreactivity scores by the Spearman’s rank correlation coefficient. Table II summarises the correlations between the IRS of hMSH2, p53 and apoptosis. Correlations of Ki-67 with hMSH2, p53 and apoptosis are represented only with regard to the PP. hMSH2 immunoreactivity positively correlated with p53 immunoreactivity ($p \leq 0.001$) with a medium strong Spearman’s rank correlation coefficient ($r_s$) of 0.449 and with apoptosis ($p=0.005$) with a low $r_s$ of 0.279. p53 immunoreactivity and apoptosis were not significantly

Figure 1. Immunohistochemistry of samples from cervical carcinoma. Magnification: x400. A) hMSH2 detection. Left: sample 89 with 20% immunoreactive tumour cells and right: sample 86 with 70% immunoreactive tumour cells. B) p53 detection. Left: sample 86 with 10% immunoreactive tumour cells and right: sample 87 with 30% immunoreactive tumour cells. C) Detection of apoptosis. Left: sample 88 with 90% immunoreactive tumour cells and right: sample 85 with 65% immunoreactive tumour cells.
Table II. Spearman’s rank correlation coefficient between hMSH2 and p53 expressions and the immunostaining of apoptosis following TUNEL assay.

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<tr>
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<th>IRS</th>
<th>PP-score</th>
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<tr>
<td>hMSH2 – p53</td>
<td>0.449* (p&lt;0.001)</td>
<td>0.507* (p&lt;0.001)</td>
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<tr>
<td>hMSH2 – apoptosis</td>
<td>0.279* (p=0.005)</td>
<td>0.190 (p=0.055)</td>
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<tr>
<td>p53 – apoptosis</td>
<td>0.178 (p=0.074)</td>
<td>0.105 (p=0.294)</td>
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<tr>
<td>hMSH2 – Ki-67</td>
<td>0.045 (p=0.658)</td>
<td>0.086 (p=0.392)</td>
</tr>
<tr>
<td>p53 – Ki-67</td>
<td>-0.027 (p=0.792)</td>
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*Statistically significant.

correlated (p=0.074). There were no significant correlations between the Ki-67 PP-score in tumour cells and the PP-scores for hMSH2 (p=0.658), p53 (p=0.392) or apoptosis (p=0.792).

Correlation of the immunoreactivity for hMSH2 and p53 proteins, and for apoptosis with clinical data of the patients. Table III shows the correlation of patient data with the IRS of hMSH2, p53 and apoptosis. No correlations were found with ER and PR status. hMSH2 and p53 immunoreactivity did not correlate with TNM stage, tumour grading or FIGO stage. For apoptosis, there were weak correlations between the IRS and T stage (r_s=0.297; p=0.003) and FIGO stage (r_s=0.262; p=0.01).

Correlation of the immunoreactivity for hMSH2 and p53 proteins and for apoptosis, with the survival rate of cervical carcinoma patients. The survival rates were analysed using the Cox’s regression model. The relative risk of death from cervical cancer was significantly increased to 1.335 for patients with increasing immunoreactivity for apoptosis (p=0.0102). The IRS for hMSH2 or p53 did not correlate with the rate of survival (Table IV).

Discussion

Correlation of the immunoreactivity for hMSH2 with p53 expression and apoptosis. Immunoreactivity for hMSH2 was significantly correlated with immunoreactivity for p53 and apoptosis. The correlation between the high expression of hMSH2 and apoptosis reflects the expected relation between the mismatch repair system and the induction of apoptosis, as demonstrated by Zhang et al., who showed that hMSH2-deficient cells do not undergo apoptosis and develop tumours after the accumulation of mutations (22). It is difficult to comment on p53 immunoreactivity, especially in the case of cervical cancer. Because of its short half life, the immunoreactivity of wild-type p53 is low and rarely detectable. High levels of p53 immunostaining are generally interpreted as abnormal nuclear accumulation of mutated p53 (30), but in cervical carcinoma the appearance of p53 mutation are reportedly rare (31). A further complication for the interpretation of p53 immunoreactivity is the fact that cervical carcinoma is strongly linked to infection with the human papilloma virus (HPV). The viral oncoprotein E6 interacts with cellular proteins and induces degradation of p53 (32). Nevertheless, we found 74% of the tumour tissues stained positively for p53, so we expect that our data reveal in most of the cases the detection of wild-type p53. While many studies presume a p53 mutation frequency of less than 20% in cervical cancer reflected in immunodiagnostic detection, other studies report higher percentages of immunodetectable p53. Spagnolletti et al. report of 48% of tissues from breast cancer expressing p53 (10), Cheah et al. detected over 70% in cervical carcinoma (31) and Haengen et al. found immunodetectable p53 in 85% in cervical carcinoma cases using the same antibody against p53 that was used in this study (33).

The correlation between immunoreactivities for hMSH2 and p53, probably wild-type p53, seems to be reasonable because hMSH2 is regulated by p53. Scherer et al. detected a response element in the promotor region of the hms2h gene (34) and Warnick et al. showed that it is active (27). Moreover, in cancer tissue, where high proliferative activity with frequent replicative errors is presumed, the mismatch repair system with hMSH2 activity is comprehensibly linked to apoptosis induction through p53 (35).

In contrast to our results, Giarnieri et al. found a correlation between a high histochemical grade of p53 and the absence of hMSH2 and hMLH1 expression in non-invasive squamous cell carcinoma of the uterine cervix (NISCC) (19). Positive staining for hMSH2 was found in 92.9% of NISCC, where only 56.5% of invasive squamous cell carcinoma of the uterine cervix (ISCC) expressed hMSH2. For ISCC the correlation of p53 overexpression and hMLH1 expression was positive.

Correlation of hMSH2 immunoreactivity with predictive tumour markers. No correlations between hMSH2 immunoreactivity and tumour grading could be detected in the 102 samples of cervical carcinoma tissues. Chung et al. (36) investigated the association of the absence of the MMR-proteins hMSH2 or hMLH1 with clinicopathological features of cervical cancer. In accordance with our study, they could not relate the MMR protein expression to any of the investigated tumour gradings, including the frequency of MSI. Giarnieri et al. report a correlation between the absence of these MMR proteins with the invasiveness of the squamous cell carcinoma of the uterine cervix. No further correlations could be found...
between MMR protein expression and histological tumour grading (19). Ciavattini et al. found the highest levels of hMSH2 and hMLH1 in preinvasive lesions compared to normal tissue and invasive squamous cervical carcinoma. The MMR is probably highly active in early cancer stages. For breast cancer, Bock et al. report a correlation between the loss of hMSH2 expression with lymph node appearance, higher degrees of malignancy and elevated proliferative activity (17).

Correlation of hMSH2, p53 and apoptosis with patient survival. The expression of hMSH2 did not correlate with the survival rate using Cox’s regression. Only the IRS for apoptosis using a TUNEL assay correlated with a lower prognosis of survival. The relation between hMSH2 expression and survival was investigated, for instance, by Chung et al., but no correlation was found (36).

Conclusion

Correlated immunoreactivities of hMSH2, p53 and apoptosis were found. Assuming p53 and hMSH2 are not mutated, then the mismatch repair processes, the activity of the cell cycle control protein, p53, and apoptosis are related processes. hMSH2 is unlikely to be a suitable prognostic factor for tumour progression or survival of patients with cervical carcinoma.

References


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