Expression of class I histone deacetylases (HDAC1 and HDAC2) in oesophageal adenocarcinomas: an immunohistochemical study

Rupert Langer,1 Kathrin Mutze,1 Karen Becker,1 Marcus Feith,2 Katja Ott,3 Heinz Höfler,4 1 and Gisela Keller1

ABSTRACT

Background Histone deacetylases (HDACs) are enzymes which play a central role in post-translational histone and non-histone protein modification. Deregulation of HDACs has been detected in various human malignancies and may also influence response to chemotherapy.

Aims To investigate the expression of class I histone deacetylase (HDAC) isoforms 1 and 2 in oesophageal adenocarcinomas.

Methods 132 primary resected tumours and 48 tumours treated by chemotherapy were analysed. Expression of HDAC1 and HDAC2 was determined by immunohistochemistry, applied on a tissue microarray and on pretherapeutic biopsies, and correlated with pathological features and prognosis.

Results There was negative or low expression of HDAC1 in 54% of tumours, moderate expression in 41% and high expression in 5%. HDAC2 expression was negative or low in 30% of tumours, moderate in 47% and high in 21%. In primary resected tumours, high HDAC2 levels were associated with lymphatic tumour spread and lower tumour differentiation grade. HDAC1 levels were not associated with pT, pN category or tumour differentiation grade. For neoadjuvant treated tumours, there was only a trend for an association with high pretherapeutic HDAC2 expression and tumour regression after chemotherapy. Pretherapeutic HDAC1 levels were not associated with regression after chemotherapy. Survival analysis failed to show any prognostic impact of HDAC1 or HDAC2 expression.

Conclusions High HDAC2 expression is associated with aggressive tumour behaviour in oesophageal adenocarcinomas. No significant prognostic value could be found with respect to overall survival or an association with response to conventional chemotherapy for HDAC expression. Immunohistochemical determination of HDACs may be useful for prediction of response to specific HDAC inhibitors.

INTRODUCTION

Locally advanced oesophageal adenocarcinoma is a highly malignant tumour, with a poor prognosis despite advances in surgery or the introduction of neoadjuvant treatment.1–5 Thus, there is a need for methods and tools that allow improvement of therapeutic approaches, for example the identification of biomarkers which may predict prognosis after resection or the response and prognosis after neoadjuvant treatment,6 or the development of alternative therapeutic strategies beyond conventional chemotherapeutic treatment.

Histone acetylation is a crucial epigenetic mechanism of the regulation of gene expression. It leads to an open chromatin structure favouring gene transcription, whereas deacetylation induces transcriptional repression through chromatin condensation.7,8 Epigenetic alterations causing aberrant gene expression are found in many tumour entities9 and are also implicated in response to chemotherapy. In addition, modulation of chromatin structure has been suggested to influence the accessibility of DNA targeting drugs such as cisplatin and thus to affect the extent of DNA damage.10,11 Besides the effects of acetylation of the chromatin structure, the function of numerous non-histone proteins can also be modified by acetylation.9

Histone deacetylases (HDACs) are enzymes involved in these chromatin modifications. They comprise four classes of proteins consisting of at least 18 HDAC isoenzymes. Among the best characterised class I isoenzymes are HDAC1 and HDAC2.7 Overexpression of HDAC1 and HDAC2 has been demonstrated in various tumour entities, such as gastric cancer,12 prostate cancer13 and renal cancer,14 with the general observation of an association between high HDAC expression and aggressive tumour behaviour. Furthermore, the development of potent class I HDAC inhibitors, which have shown potent antitumoural activity both in preclinical experiments and in clinical trials, has gained increasing attention towards HDAC expression studies in vitro and in/ex vivo.15,16 HDAC expression has not been investigated in adenocarcinomas of the oesophagus so far. In this study, we thus aimed to evaluate the diagnostic, prognostic and predictive impact of HDAC1 and HDAC2 expression in oesophageal adenocarcinomas by immunohistochemistry. For assessment of the distribution and the prognostic impact of HDAC1 and HDAC2 expression, primary resected carcinomas were investigated. For the determination of an association between HDAC1 and HDAC2 expression and response to conventional chemotherapy, we analysed pretherapeutic biopsies of oesophageal adenocarcinoma patients treated by platinum/5-fluorouracil (5-FU) based neoadjuvant chemotherapy and correlated the HDAC1 and HDAC2 expression patterns with histopathological tumour regression after treatment.

MATERIALS AND METHODS

Patients

Paraffin-embedded tumour samples from 179 patients with oesophageal adenocarcinoma, who
were treated between 1991 and 2006 in the Department of Surgery of the Klinikum Rechts der Isar der Technischen Universität München were investigated. All patients gave consent for additional molecular analyses at the time of their original operation. Patient age ranged from 33 years to 83 years. The female/male ratio was 15/164.

The study group consisted of two subgroups. The first group of 131 patients had been treated by radical surgical resection—either transthoracic or transhiatal oesophagectomy—without neoadjuvant chemotherapy or radiochemotherapy. The mean overall survival for these patients, calculated from the day of surgery, was 73.0 months (95% CI 57 to 88 months) until last contact or death. The pT category (according to UICC 2010) was as follows: pT1, 58 cases (44%); pT2, 24 cases (18%); and pT3–4, 49 cases (37%). Lymph node metastases were present in 57 cases (41.6%). Tumour grading was G1 (well differentiated) in 57 cases (41.6%), G2 (moderately differentiated) in 54 cases (45%), and G3–G4 (poorly differentiated) in 62 cases (48%).

A second group, 48 patients with locally advanced carcinomas (cT3–T4) were treated with a conventional, 5-FU and cisplatin based chemotherapy. Histopathological response evaluation of the tumours was performed as previously described: patients with 50% residual tumour or less after treatment (tumour regression grades 1 and 2) were classified as responders (n=25; 45%); patients with more than 50% residual tumour (tumour regression grade 3) were classified as non-responders (n=25; 55%). Mean overall survival, which was calculated from the first day of chemotherapy until last contact or death was 57.4 months (95% CI 24.7 to 50.1) for all patients. Responders had an improved survival (p=0.2), with a median overall survival of 45.8 months (95% CI 25 to 67 months) compared to non-responders who had a mean overall survival of 25.5 months (95% CI 18 to 35 months).

**Immunohistochemistry**

Immunohistochemistry was performed on formalin-fixed and paraffin-embedded (FFPE) tissue. For the analysis of primary resected carcinomas immunohistochemical stainings were applied on a tissue microarray, which consisted of samples of resected carcinomas immunohistochemical stainings were given in crosstabs and were evaluated with the χ² test. Survival analysis was performed using Kaplan–Meier estimates, log rank tests and Cox’s proportional hazards regression analysis. All tests were two-sided, and the significance level was set at 5%.

**RESULTS**

**Distribution of HDAC1/2 expression in oesophageal adenocarcinomas**

According to the criteria for reliable immunohistochemical staining, described above, 178 cases were available for HDAC1 expression analysis and 155 cases for HDAC2 expression analysis. In total, 93 tumours showed no or low HDAC1 expression (54%), 71 tumours (41%) had a moderate HDAC1 expression and 9 tumours (5%) had high HDAC1 expression. HDAC2 expression was low/negative in 49 tumours (32%), moderate in 73 tumours (47%) and high in 33 (21%) of the cases.

**Correlation of HDAC1/2 expression with clinicopathological parameters and prognosis in primary resected tumours**

For the determination of an association between HDAC expression and clinicopathological parameters and patient survival, primary resected tumours were analysed.

HDAC1 expression, which was evaluable for 126 tumours, was neither associated with tumour category (UICC pT category), nor presence of lymph node metastases (UICC pN category), distant metastases (UICC p/M category) and tumour differentiation grade. HDAC2 expression was determined in 115 tumour spots of the tissue microarray. In these cases, presence of HDAC2 expression showed no correlation with tumour stage, lymph node or distance metastases and grading. However, high HDAC2 levels were associated with lymphatic tumour spread (pN category; p=0.046) and lower tumour differentiation grade (p=0.002). Survival analysis failed to show any prognostic impact of HDAC1 or HDAC2 expression (table 1).

**HDAC1/2 expression and response to neoadjuvant chemotherapy**

For the investigation of an association between HDAC expression and response to neoadjuvant chemotherapy, HDAC1 and HDAC2 staining was determined on paraffin blocks.

HDAC1 expression was evaluated in 126 tumours, and was neither associated with tumour category (UICC pT category), nor presence of lymph node metastases (UICC pN category), distant metastases (UICC p/M category) and tumour differentiation grade. HDAC2 expression was determined in 115 tumour spots of the tissue microarray. In these cases, presence of HDAC2 expression showed no correlation with tumour stage, lymph node or distance metastases and grading. However, high HDAC2 levels were associated with lymphatic tumour spread (pN category; p=0.046) and lower tumour differentiation grade (p=0.002). Survival analysis failed to show any prognostic impact of HDAC1 or HDAC2 expression (table 1).
having low and one high pretherapeutic tumour HDAC2 expression. Patients’ overall survival was not correlated with HDAC1 and HDAC2 expression (table 2).

DISCUSSION

We present, to our knowledge, the first immunohistochemical study of the expression of class I HDAC isoforms 1 and 2 in oesophageal adenocarcinoma. A moderate or high HDAC expression could be detected in 46% of the cases for HDAC1 and 70% of the cases for HDAC2. These cases can be considered as being ‘HDAC positive’ according to Weichert and coworkers who have analysed HDAC expression in a large collective of various human malignancies, such as gastric cancer,12 prostate cancer,13 colorectal cancer23 or renal cancer.14 Our results are in line with findings of these studies, with rates of 30–65% HDAC positive tumours being described.9

In general, high HDAC expression is considered to be associated with aggressive tumour behaviour. In vitro studies have shown that high HDAC activity leads to tumour dedifferentiation and enhanced tumour cell proliferation.24 In our study, high HDAC2 levels were associated with the presence of lymph node

Table 1  Histone deacetylase isoforms 1 and 2 (HDAC1/2) expression in primary resected tumours

<table>
<thead>
<tr>
<th>HDAC1</th>
<th>HDAC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative/low</td>
<td>Moderate</td>
</tr>
<tr>
<td>pT category</td>
<td>0.83</td>
</tr>
<tr>
<td>pT1</td>
<td>27</td>
</tr>
<tr>
<td>pT2</td>
<td>13</td>
</tr>
<tr>
<td>pT3–pT4</td>
<td>23</td>
</tr>
<tr>
<td>pN category</td>
<td>0.267</td>
</tr>
<tr>
<td>pN0</td>
<td>32</td>
</tr>
<tr>
<td>pN1–3</td>
<td>31</td>
</tr>
<tr>
<td>Tumour differentiation</td>
<td>0.098</td>
</tr>
<tr>
<td>Well/moderate</td>
<td>26</td>
</tr>
<tr>
<td>Poor</td>
<td>37</td>
</tr>
</tbody>
</table>

HDAC1 and HDAC2 expression in primary resected oesophageal adenocarcinomas. Correlation with pathological parameters UICC pT- category, pN category and tumour differentiation ( grading). (χ² testing: * for high HDAC expression vs negative/low/moderate.)

Figure 1 Examples of immunohistochemical staining for histone deacetylase isoform 1 (HDAC1) expression (A, low expression; B, moderate expression; C, high expression) and HDAC2 expression (D, low expression; E, moderate expression; F, high expression) in adenocarcinomas of the oesophagus (original magnification ×200).
metastases and lower tumour differentiation grade in primary resected tumours. Similar results were reported by Weichert et al for gastric cancer, where high HDAC1 and HDAC2 expression is also associated with nodal tumour spread and with a worse patient outcome. Other studies report a correlation of high expression levels of class I HDACs with tumour dedifferentiation and higher proliferation in prostate carcinoma, or a negative impact of HDAC2 expression on patient prognosis in colorectal carcinoma. In breast cancer, more aggressive tumours have been shown to express higher HDAC1 levels. In contrast, Toh et al have found an association between decreased HDAC1 expression and advanced tumour stages in oesophageal squamous cell carcinomas. In our study, high HDAC2 expression showed a trend for an association with poor survival, but we could not demonstrate a significant independent prognostic value for HDAC expression in oesophageal adenocarcinoma. Therefore, high HDAC expression may represent a surrogate marker for aggressive tumour behaviour in oesophageal adenocarcinoma rather than being an independent prognostic factor.

HDAC expression may also have an impact on tumour response to conventional chemotherapeutic drugs. The accessibility of DNA targeting drugs such as cisplatin may be influenced by modulation of chromatin structure and thus may affect the extent of the DNA damage. We aimed to investigate a potential influence of HDAC expression on chemotherapy response to a neoadjuvant, cisplatin and 5-FU based chemotherapy in oesophageal adenocarcinoma. For that purpose, we correlated HDAC1 and HDAC2 expression in pretherapeutic biopsies with histopathological tumour regression after chemotherapy. We observed only a non-significant trend for an association of high HDAC2 expression with a better chemotherapy response, so that the predictive value of determination of HDAC expression with regard to response to conventional chemotherapy may be disregarded, although the study has limitations due to a relatively small sample size.

In the recent past, the inhibition of HDAC by siRNA knockdown or by specific HDAC inhibitors (HDI) has been shown to suppress tumour growth in vitro and in vivo. Substances like hydroxamic acids such as suberoylanilide hydroxamic acid (vorinostat), or short-chain fatty acids such as valproic acid, which are targeting class I isoforms HDAC1, HDAC2, have entered late-phase clinical trials for the treatment of haematological and solid malignancies including colorectal cancer and gastric cancer. Most recently, vorinostat has been approved by the US Food and Drug Administration (FDA) for the treatment of patients with cutaneous T cell lymphoma (Olsen 2007). Moreover, development of novel HDIs like resminostat (RAS2410) is an ongoing process.

Despite their antitumoural potential, synergistic effects of HDIs and conventional chemotherapeutics, especially DNA affecting drugs like cisplatin, have been proposed. HDIs have been shown to act as radiosensitisers in a variety of cancer cell lines, including colon and ovary cancer cells, so HDIs might be extremely useful for chemotherapeutic or radiochemotherapeutic combination therapies. This may be of major importance particularly for oesophageal adenocarcinoma, where there is a considerable rate of non-responders to conventional neoadjuvant chemotherapy. Given the relatively high rate of tumours which show class I HDAC1 expression, HDAC inhibition may represent a potent alternative therapeutic option for oesophageal adenocarcinoma patients.

In conclusion, we have shown that high class I HDAC expression and response to neoadjuvant chemotherapy. HDAC1/2 expression and response to neoadjuvant chemotherapy in oesophageal adenocarcinomas. Correlation with histopathological tumour regression after chemotherapy. (χ^2 testing * for high HDAC expression vs negative/low/moderate.)

### Table 2 HDAC1/2 expression and response to neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>HDAC1</th>
<th>Negative/low (n=30)</th>
<th>Moderate (n=13)</th>
<th>High (n=4)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>15</td>
<td>6</td>
<td>2</td>
<td>0.80</td>
</tr>
<tr>
<td>Non-responders</td>
<td>15</td>
<td>7</td>
<td>2</td>
<td>0.16</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>HDAC2</th>
<th>Negative/low (n=9)</th>
<th>Moderate (n=13)</th>
<th>High (n=15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>0.07*</td>
</tr>
<tr>
<td>Non-responders</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

**Take-home messages**

- Histone deacetylase isoform 1 (HDAC1) expression can be detected in 46% and HDAC2 expression in 68% of primary resected oesophageal adenocarcinomas.
- High HDAC2 expression is associated with the presence of lymph node metastases and lower tumour differentiation grade in primary resected tumours, reflecting an association with a more aggressive tumour behaviour.
- There is no significant association between HDAC1 and HDAC2 expression and response to neoadjuvant chemotherapy in oesophageal adenocarcinomas.

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**Competing interests** None.
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