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,[1,4] Gisela Keller[1]

[1]Institute of Pathology, Technische Universität München, Munich, Germany
[2]Department of Surgery, Technische Universität München, Munich, Germany
[3]Department of Surgery, University of Heidelberg, Heidelberg, Germany
[4]Institute of Pathology, Helmholtz-Zentrum München, Oberschleissheim, Germany

Correspondence to
Rupert Langer, Institute of Pathology, Technische Universität München, Trogerstr. 18, D-81675 Munich, Germany; rupert.langer@lrz.tum.de

RL and KM contributed equally to this work.

Accepted 4 August 2010
Published Online First 5 October 2010

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 entities[9] 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.[8]

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 cancer[13] 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[17]) 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 9 cases (7%), 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.[18 19] Histopathological response evaluation of the tumours was performed as previously described[20 21]: patients with 50% residual tumour or less after treatment (tumour regression grades 1 and 2) were classified as responders (n=23; 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 37.4 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 25.3 months (95% CI 18 to 33 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 131 tumours and was constructed as described previously.[22] For the analysis of an association of HDAC expression with response to neoadjuvant treatment, sections of 48 pretherapeutic tumour biopsies were analysed and the results compared with tumour regression as described above.

The paraffin blocks were freshly cut (3 μm). Subsequent to heat-induced antigen retrieval using 10 mM citrate buffer, pH 6, the sections were incubated with antibodies for HDAC1 (rabbit polyclonal prediluted; dilution 1:10; Abcam, Cambridge, UK) or HDAC2 (mouse monoclonal; dilution 1:200 000; Abcam) followed by secondary biotinylated antibody. Immunodetection was performed with the Dako REALTM Detection system peroxidase/DAB+ kit (DAKO, Glostrup, Denmark). Appropriate positive and negative controls were included in each reaction.

HDAC1/HDAC2 expression was assessed based on the intensity of nuclear immunostaining and the percentage of positive tumour cells. For a reliable evaluation of immunohistochemical staining an amount of 100 tumour cells per spot or slide, respectively, was required. The intensity was scored as 0 (no immunostaining), 1 (weak immunostaining), 2 (moderate immunostaining) or 3 (strong immunostaining). The percentage of nuclear positive tumour cells was scored as 0 (none), 1 (<10%), 2 (10–50%), 3 (51–80%) or 4 (>80%). Multiplication of the scores for intensity and percentage resulted in a staining index ranging from 0 to 12. A staining index of 0–6 was defined as negative/weak ('negative'), and a staining index of 8–12 as moderate/high ('positive') for HDAC1 and HDAC2 expression, according to the evaluation system which was described by Weichert *et al*[12]. In addition, tumours with a staining index of >9 were subclassified as 'high HDAC1/2 expressing'. The evaluation of immunohistochemical staining was performed by two independent observers (KM, RL). Differences were discussed at a double-header microscope to gain a final consensus (see figure 1).

Statistical analysis

SPSS V.17.0 was used for statistical analysis. Associations between immunohistochemical expression patterns and pathological features were given in crosstabs and were evaluated with the χ^2 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, 173 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/cM 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 pretherapeutic biopsies and the expression patterns were correlated with histopathological tumour regression after treatment. A total of 47 tumours were evaluable for HDAC1 expression analysis according to the criteria described above, and there was no association between pretherapeutic expression and tumour regression after chemotherapy. HDAC2 expression was evaluated in 37 cases only due to less or no amount of tumour tissue in the paraffin blocks. Here, there was only a non-significant trend for an association with improved chemotherapy response (p=0.07) in 37 patients. Of note, in the two patients with complete tumour regression (tumour regression grade 1), there was one showing low and one moderate pretherapeutic HDAC1 tumour expression, and one

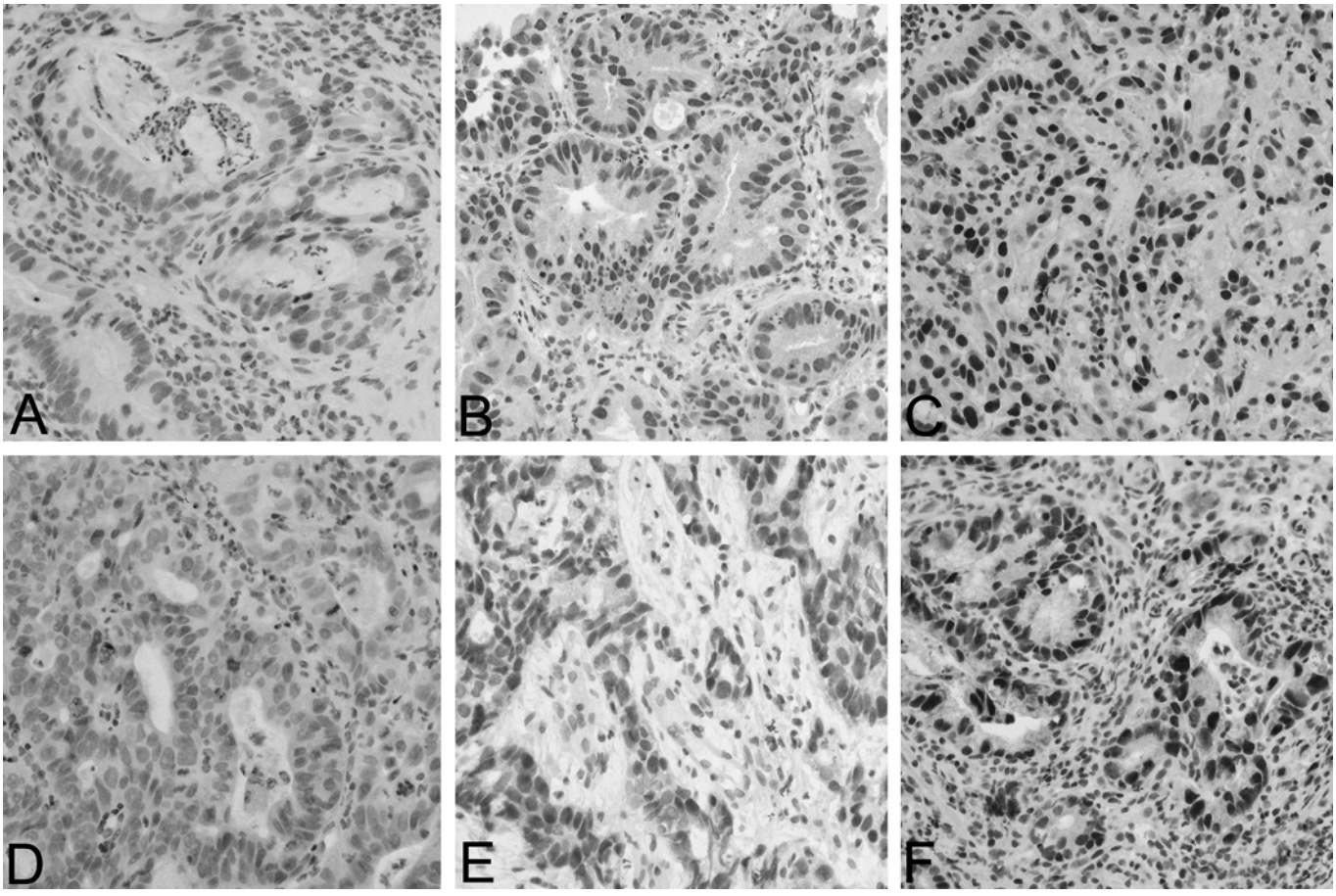

Figure 1 Examples of immunohistochemical staining for histone deacetylase isoform 1 (HDAC1) expression (A, low expression; B, moderate expression; C, high expression) and HDAC2 expression (D, low expression; E, moderate expression; F, high expression) in adenocarcinomas of the oesophagus (original magnification ×200).

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 cancer[23] 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

	HDAC1				HDAC2			
	Negative/low (n=63)	Moderate (n=58)	High (n=5)	p Value	Negative/low (n=37)	Moderate (n=60)	High (n=18)	p Value
pT category				0.83				0.204
pT1	27	26	3		18	27	5	
pT2	13	10	0		3	14	4	
pT3–pT4	23	22	2		16	19	9	
pN category				0.267				0.131
pN0	32	37	2		21	36	6	(0.046*)
pN1–3	31	21	3		16	24	12	
Tumour differentiation				0.098				0.009
Well/moderate	26	35	2		21	33	3	(0.002*)
Poor	37	23	3		16	27	15	

HDAC1 and HDAC2 expression in primary resected oesophageal adenocarcinomas. Correlation with pathological parameters UICC pT- category, pN category and tumour differentiation (grading). (χ^2 testing; *for high HDAC expression vs negative/low/moderate.)

Table 2 HDAC1/2 expression and response to neoadjuvant chemotherapy

	HDAC1				HDAC2			
	Negative/low (n=30)	Moderate (n=13)	High (n=4)	p Value	Negative/low (n=9)	Moderate (n=13)	High (n=15)	p Value
Chemotherapy response				0.80				0.16
Responders	15	6	2		4	4	10	(0.07*)
Non-responders	15	7	2		5	9	5	

HDAC1 and HDAC2 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.)

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.[12] Other studies report a correlation of high expression levels of class I HDACs with tumour dedifferentiation and higher proliferation in prostate carcinoma,[13] or a negative impact of HDAC2 expression on patient prognosis in colorectal carcinoma.[23] In breast cancer, more aggressive tumours have been shown to express higher HDAC I levels.[25] In contrast, Toh *et al* have found an association between decreased HDAC1 expression and advanced tumour stages in oesophageal squamous cell carcinomas.[26] 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.[27 28] 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[29 30] or by specific HDAC inhibitors (HDI) has been shown to suppress tumour growth in vitro and in vivo.[31 32] 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[33] and solid malignancies including colorectal cancer and gastric cancer.[12] 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).[34] Moreover, development of novel HDIs like resminostat (RAS2410) is an ongoing process.[11]

Despite their antitumoural potential, synergistic effects of HDIs and conventional chemotherapeutics, especially DNA affecting drugs like cisplatin, have been proposed.[11] HDIs have been shown to act as radiosensitisers in a variety of cancer cell lines, including colon and ovary cancer cells,[35] 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.[21] 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.

However, at present there are virtually no reliable data as to whether HDAC expression in a given tumour entity might predict the therapeutic response to HDI.[36] It may be probable—although this is speculative—that treatment response is especially prominent in tumours with a strong HDAC expression. Since HDI have already entered clinical practice there is an urgent need to identify biomarkers to stratify both patients and tumours into subgroups that are responsive and likely to undergo clinical benefit and those who are not.

In conclusion, we have shown that high class I HDAC expression, especially HDAC2 expression, is associated with aggressive tumour behaviour in adenocarcinomas of the oesophagus, reflected by the association with lower tumour differentiation and lymphatic tumour spread. We could not demonstrate an independent significant prognostic value with regard to patient survival or an association with response to conventional chemotherapy for HDAC expression. Further studies will be necessary to determine the predictive value of HDAC expression with regard to response to specific HDIs or an enhancement of the effect of conventional chemotherapy by an additional application of HDIs.

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.

Acknowledgements The authors thank Mrs Andrea Brütting for excellent technical assistance.

Funding This work was in part supported by the Wilhelm-Sander-Stiftung (grant number 2006.035-1 to GK and KO) and the Deutsche Krebshilfe (grant number 70-2789-Si3 to HH).

Competing interests None.

Ethics approval This study was conducted with the approval of the Ethikkommission Medizinische Fakultät TU München.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Brown LM,** Devesa SS, Chow WH. Incidence of adenocarcinoma of the oesophagus among white Americans by sex, stage, and age. *J Natl Cancer Inst* 2008;**100**:1184—7.
2. **DeMeester SR.** Adenocarcinoma of the esophagus and cardia: a review of the disease and its treatment. *Ann Surg Oncol* 2006;**13**:12—30.
3. **Jemal A,** Siegel R, Ward E, *et al*. Cancer statistics, 2009. *CA Cancer J Clin* 2009;**59**:225—49.
4. **Siewert JR,** Lordick F, Ott K, *et al*. Induction chemotherapy in Barrett cancer: influence on surgical risk and outcome. *Ann Surg* 2007;**246**:624—8; discussion 628—31.
5. **Cunningham D,** Allum WH, Stenning SP, *et al*. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006;**355**:11—20.
6. **Fareed KR,** Kaye P, Soomro IN, *et al*. Biomarkers of response to therapy in oesophago-gastric cancer. *Gut* 2009;**58**:127—43.
7. **Glozak MA,** Seto E. Histone deacetylases and cancer. *Oncogene* 2007;**26**:5420—32.
8. **Kristensen LS,** Nielsen HM, Hansen LL. Epigenetics and cancer treatment. *Eur J Pharmacol* 2009;**625**:131—42.
9. **Weichert W.** HDAC expression and clinical prognosis in human malignancies. *Cancer Lett* 2009;**280**:168—76.
10. **Davies NP,** Hardman LC, Murray V. The effect of chromatin structure on cisplatin damage in intact human cells. *Nucleic Acids Res* 2000;**28**:2954—8.
11. **Kim MS,** Blake M, Baek JH, *et al*. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res* 2003;**63**:7291—300.
12. **Weichert W,** Roske A, Gekeler V, *et al*. Association of patterns of class I histone deacetylase expression with patient prognosis in gastric cancer: a retrospective analysis. *Lancet Oncol* 2008;**9**:139—48.
13. **Weichert W,** Roske A, Gekeler V, *et al*. Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. *Br J Cancer* 2008;**98**:604—10.
14. **Fritzsche FR,** Weichert W, Roske A, *et al*. Class I histone deacetylases 1, 2 and 3 are highly expressed in renal cell cancer. *BMC Cancer* 2008;**8**:381.
15. **Botrugno OA,** Santoro F, Minucci S. Histone deacetylase inhibitors as a new weapon in the arsenal of differentiation therapies of cancer. *Cancer Lett* 2009;**280**:134—44.
16. **Minucci S,** Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 2006;**6**:38—51.
17. **Sobin L,** Gospodarowicz ML, Wittekind Ch. *TNM classification of malignant tumors*. New York: John Wiley & Sons, 2010.
18. **Lordick F,** Ott K, Krause BJ, *et al*. PET to assess early metabolic response and to guide treatment of adenocarcinoma of the oesophagogastric junction: the MUNICON phase II trial. *Lancet Oncol* 2007;**8**:797—805.
19. **Lordick F,** Stein HJ, Peschel C, *et al*. Neoadjuvant therapy for oesophagogastric cancer. *Br J Surg* 2004;**91**:540—51.
20. **Becker K,** Mueller JD, Schulmacher C, *et al*. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. *Cancer* 2003;**98**:1521—30.
21. **Langer R,** Ott K, Feith M, *et al*. Prognostic significance of histopathological tumor regression after neoadjuvant chemotherapy in esophageal adenocarcinomas. *Mod Pathol* 2009;**22**:1555—63.
22. **Langer R,** Von Rahden BH, Nahrig J, *et al*. Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study. *J Clin Pathol* 2006;**59**:631—4.
23. **Weichert W,** Roske A, Niesporek S, *et al*. Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo. *Clin Cancer Res* 2008;**14**:1669—77.
24. **Munster PN,** Troso-Sandoval T, Rosen N, *et al*. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. *Cancer Res* 2001;**61**:8492—7.
25. **Suzuki J,** Chen YY, Scott GK, *et al*. Protein acetylation and histone deacetylase expression associated with malignant breast cancer progression. *Clin Cancer Res* 2009;**15**:3163—71.
26. **Toh Y,** Yamamoto M, Endo K, *et al*. Histone H4 acetylation and histone deacetylase 1 expression in esophageal squamous cell carcinoma. *Oncol Rep* 2003;**10**:333—8.
27. **Jones PA,** Baylin SB. The epigenomics of cancer. *Cell* 2007;**128**:683—92.
28. **Napieralski R,** Ott K, Kremer M, *et al*. Methylation of tumor-related genes in neoadjuvant-treated gastric cancer: relation to therapy response and clinicopathologic and molecular features. *Clin Cancer Res* 2007;**13**: 5095—102.
29. **Bolden JE,** Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 2006;**5**:769—84.
30. **Wilson AJ,** Byun DS, Popova N, *et al*. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. *J Biol Chem* 2006;**281**:13548—58.
31. **Marks P,** Rifkind RA, Richon VM, *et al*. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer* 2001;**1**:194—202.
32. **Stimson L,** Wood V, Khan O, *et al*. HDAC inhibitor-based therapies and haematological malignancy. *Ann Oncol* 2009;**20**:1293—302.
33. **Mandl-Weber S,** Meinel F, Jankowsky R, *et al*. The novel inhibitor of histone deacetylase resminostat (RAS2410) inhibits proliferation and induces apoptosis in multiple myeloma (MM) cells. *Br J Haematol* 2010;**149**:518—28.
34. **Olsen EA,** Kim YH, Kuzel TM, *et al*. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2007;**25**:3109—15.
35. **Lin CT,** Lai HC, Lee HY, *et al*. Valproic acid resensitizes cisplatin-resistant ovarian cancer cells. *Cancer Sci* 2008;**99**:1218—26.
36. **Stimson L,** La Thangue NB. Biomarkers for predicting clinical responses to HDAC inhibitors. *Cancer Lett* 2009;**280**:177—83.

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

Rupert Langer, Kathrin Mutze, Karen Becker, Marcus Feith, Katja Ott, Heinz Höfler and Gisela Keller

J Clin Pathol 2010 63: 994-998 originally published online October 5, 2010
doi: 10.1136/jcp.2010.080952