Phenotypic Impact of Deregulated Expression of Class I Histone Deacetylases in Urothelial Cell Carcinoma of the Bladder

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Deregulated expression of histone deacetylases (HDACs) has been implicated in tumorigenesis. Herein, we investigated class I HDACs expression in bladder urothelial cell carcinoma (BUCC), its prognostic value and biological significance. Significantly increased transcript levels of all HDACs were found in BUCC compared to 20 normal mucosas, and these were higher in lower grade and stage tumors. Increased HDAC3 levels were associated with improved patient survival. SiRNA experiments showed decrease cell viability and motility, and increased apoptosis. We concluded that class I HDACs play an important role in bladder carcinogenesis through deregulation of proliferation, migration and apoptosis, constituting putative therapeutic targets. © 2013 Wiley Periodicals, Inc.

Key words: bladder cancer; class I HDACs; apoptosis; viability

INTRODUCTION

Bladder cancer is a global health concern, being the 11th most common cancer in both genders, accounting for 386 300 new cases and 150 200 deaths per year, occurring mostly in the 7th decade of life [1,2]. The adjusted incidence is approximately four times higher in males than in females, although mortality is only about twice in men compared to women [2]. Among bladder tumors, urothelial (transitional cell) carcinoma (BUCC) is the most frequent histological subtype, comprising 90% of all cases [3]. Commonly, urothelial carcinomas are divided in two major groups, the noninvasive or early invasive tumors [confined to the urothelium (CIS, Ta) or to the lamina propria (T1), respectively] also known as "superficial BUCC," whereas the remainder are deeply invasive (i.e., infiltrating the muscularis propria and beyond) cancers (T2–T4). The noninvasive tumors are more prevalent, less aggressive, yet with a high rate of recurrence, while invasive tumors are less common, but much more clinically aggressive [4,5]. The histology of infiltrating urothelial carcinomas is variable, although most pT1 tumors are papillary, low or high grade, and most T2–T4 carcinomas are nonpapillary and high grade [6].

BUCC is a very heterogeneous disease and there is substantial evidence for the existence of two distinct molecular pathways in bladder carcinogenesis, in which distinct genetic alterations are responsible for the formation of noninvasive or invasive urothelial tumors, resulting in divergent biological and clinical phenotypes [7–9]. Noninvasive carcinomas typically arise in the context of hyperplastic urothelium and harbor oncogene mutations, such as in fibroblast growth factor receptor 3 (*FGFR3*), whereas invasive tumors arise through dysplasia and often display mutations in tumor suppressor genes, such as

TP53 [10–12]. At an epigenetic level, these two groups of tumors are also distinct. Invasive tumors depict higher levels of aberrant methylation and upregulation of many miRNAs, whereas, in contrast, noninvasive tumors display low levels of aberrant hypermethylation and downregulation of miRNAs [13,14].

Histone posttranslational modifications play a crucial role in chromatin structure, being acetylation the most extensively characterized [15]. Histones' acetylation is a dynamic process controlled by the antagonistic actions of acetyltransferases (HATs) and deacetylases (HDACs), which maintain the equilibrium of acetyl groups added or removed from lysine residues, respectively [16]. HDACs are a family of 18 genes, that act as co-repressors promoting chromatin compaction, which are grouped into four classes depending on amino acid sequence homology in the catalytic domain [17]. Class I HDACs consists on ubiquitously expressed nuclear enzymes, comprising HDAC1, HDAC2, HDAC3, and HDAC8, which are implicated in the regulation of cell differentiation, proliferation, cell-cycle progression, and apoptosis [18]. Except for HDAC8, they are found as subunits of several multiprotein co-repressor complexes and interact with various transcription factors, being HDAC1 and HDAC2 present in the same complexes (Sin3, NuRD, and CoREST) as homo- or heterodimers [19,20].

Aberrant expression of class I HDACs has been reported in several human cancers, including colorectal, gastric, and prostate, and some of these studies have already disclosed their participation in different cell functions frequently deregulated in tumors [21–23]. Nevertheless, the role of these enzymes in bladder carcinogenesis remains elusive. Hence, we aimed to characterize the expression patterns of each member of class I HDACs in bladder cancer and evaluate their prognostic value, through correlation of molecular findings with standard clinicopathological data. Moreover, the biological role of altered class I HDACs was investigated using a BUCC cell line as an in vitro model.

MATERIALS AND METHODS

Patients and Samples

The 127 BUCC samples selected for this study correspond to a series of patients diagnosed and primarily treated with radical cystectomy or transurethral resection, between 1992 and 2011 at Portuguese Oncology Institute—Porto, Portugal, of which fresh frozen tissue samples were available. For control purposes, 20 morphological normal bladder mucosa (NB) tissues were obtained from patients with prostate cancer submitted to radical prostatectomy. All specimens were fresh-frozen at −80°C and subsequently cut in a cryostat for nucleic acid and protein extraction. From each specimen, fragments were routinely collected, formalin-fixed, and paraffin-

embedded for routine histopathological examination, including grade and pathological staging, by an expert pathologist. Relevant clinical data was collected from the clinical charts. This study was approved by the institutional review board (Comissão de Ética para a Saúde).

Real-Time Quantitative PCR (qRT-PCR)

RNA was extracted from tissues and cell lines using TRIzol® (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. First strand synthesis was performed using the high-capacity cDNA Reverse Transcription Kit from Applied Biosystems (Foster City, CA). Expression of target genes was quantified using Taqman probes, acquired as predeveloped assays from Applied Biosystems [HDAC1 (Hs02621185_s1), HDAC2 (Hs00231032_m1), HDAC3 (Hs00187320_m1), and HDAC8 (Hs00218503_m1)] and normalized to the expression of HPRT (Hs01003267_m1), a housekeeping gene.

Western Blot

Whole cell line protein extraction was performed using complete RIPA buffer (Santa Cruz Inc., Santa Cruz, CA) and protein from tissues was extracted using TRIzol® Reagent (Invitrogen) according to manufacturer's instructions. Protein extract concentrations were determined using Qubit® 2.0 Fluorometer (Applied Biosystems). Subsequently, 30 μg of total protein were loaded in each well, and separated by SDS–PAGE, transferred to nitrocellulose membranes and probed with antibodies against HDAC1 (Sigma-Aldrich, Schnelldorf, Germany, 1:1000), HDAC2 (Abcam, Cambridge, UK, 1:6000), HDAC3 (Abcam, 1:6000), HDAC8 (Abcam, 1:1000), p21 (BD Pharmingen™, Franklin Lakes, NJ, 1:500) or the endogenous control β-actin (Sigma-Aldrich, 1:8000). Secondary antibodies, conjugated with horseradish peroxidase, were incubated at a dilution of 1:3000. Finally, blots were developed using Immun-Star™ WesternC™ Kit according to manufacturer's indications (BioRad, Hercules, CA) and exposed to Amersham Hyperfilm (GE Healthcare, Fairfield, CT). Relative optical density determination was performed using QuantityOne® Software version 4.6.6. (BioRad). For cell lines, three independent experiments were performed.

Cell Culture

Four urothelial BUCC cell lines [5637, J82, T24, TCCSUP (ATCC—American Type Culture Collection, Rockville, MD, USA)] were grown in order to select the most suitable for in vitro studies. All BUCC cell lines were cultured in the recommended medium, supplemented with 10% fetal bovine serum (FBS) (GIBCO®, Invitrogen) and 1% penicillin–streptomycin (P-S) (GIBCO®, Invitrogen) at 37°C in a humidified atmosphere containing 5% CO_2, and were tested for Mycoplasma spp. contamination (PCR Mycoplasma

Detection Set, Clontech Laboratories, Oxford, UK). After expression analysis for of each class I HDACs, the 5637 cell line was chosen for further studies (data not shown).

Transient Transfection

One day prior to transfection, 5637 cells were seeded under standard conditions in 6-well and 96-well flat-bottomed culture plates in order to reach 30–50% confluence. Two sets of double-stranded small interference RNA (siRNA) for HDAC1, 2, 3, and 8 and the silencer-negative siRNA as a control were purchased from Eurofins MWG (Ebersberg, Germany), purified and desalted. The sense strands of the HDAC siRNA sequences utilized were as follows: HDAC1, AAGCA-GAUGCAGAGAUUCAAC and CUGUACAUUGACAU UGAUA; HDAC2, AACAGACGUUAAGGAAGAA and GGAUUACAUCAUGCUAAGA; HDAC3, GGCACC-CAAUGAGUUCUAU and GGCUUCACCAAGAGU-CUUA; HDAC8, CAUUCAGGAUGGCAUACAA and GUCCCGAGUAUGUCAGUAU. Cells were transfected with siRNA (100 nM) using Oligofectamine (Invitrogen), as indicated by the manufacturer. Cells were then collected for further investigation 72 h after transfection. Silencing was validated by qRT-PCR and Western blot.

Viability Assay

Cell viability of 5637 cells following 24-, 48-, and 72-h treatment with class I HDACs siRNAs, performed in 96-well flat-bottomed culture plates at 12 000 cells per well, was evaluated by incorporation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) (Sigma-Aldrich). The absorbance was measured using a microplate reader (FLUOstar Omega, BMG Labtech, Offenburg, Germany) at a wavelength of 540 nm with background subtraction at 630 nm. Three replicates were performed for each condition, using triplicates for each experiment.

Apoptosis Assay

Cell apoptosis was quantified using APO Percentage apoptosis assay kit (Biocolor Ltd, Belfast, Northern Ireland) according to the manufacturer's instructions. The assay was performed with the same cell conditions of the MTT assay, with apoptotic cells measured at the end of 72 h. The absorbance was determined using a microplate reader (FLUOstar Omega, BMG Labtech) at a wavelength of 550 nm with background subtraction at 620 nm. Three independent experiments were performed, using six replicates for each experiment.

Wound Healing Assay

Cell migration of 5637 cells was examined using a monolayer wounding method, after 48 h of transfection, performed in six-well flat-bottomed culture plates initially seeded with 500 000 cells per well. The monolayer was wounded by scraping a line across the well with a sterile pipette tip. Cells were washed with PBS and refreshed with RPMI supplemented with 10% fetal bovine serum. After 0, 9, and 24 h, the cultured cells were observed under a phase-contrast microscope (Olympus IX51, Olympus, UK) and photographed at marked spots. Three replicates were performed for each condition.

Statistical Analysis

Differences in quantitative expression levels between BUCC and NB were assessed using the nonparametric Mann–Whitney (M-W) U-test. The relationship between expression ratios and other standard clinicopathological variables (gender, tumor stage, and grade) were determined using the M-W or Kruskall–Wallis (K-W) tests, as appropriate. A Spearman nonparametric correlation test was additionally performed to compare age and expression levels. The results of all functional in vitro assays (scramble vs. silenced cells) were analyzed using one-way analysis of variance (ANOVA), complemented with a post hoc Dunnet's test for multiple comparisons, when appropriate.

To test the prognostic significance of the expression status of each class I HDAC, samples were categorized into two groups based on the respective expression levels (using the median as the cutoff value). Disease-specific (DSS) and disease-free survival (DFS) curves were then constructed for each HDAC using the Kaplan–Meier method and groups survival were compared using log-rank test. Since follow-up time was very heterogeneous, analysis was limited to the first 5 yr, censoring all times that exceeded that period. Hazard ratios (HR) were estimated using univariate Cox regression. A Cox-regression model comprising all clinicopathological variables and molecular variables which reached statistical significance in univariate analysis (multivariate test) was also constructed.

All analyses were performed with SPSS software (SPSS Version 20.0, Chicago, IL) and statistical significance was set at $P < 0.05$.

RESULTS

Clinical and Pathological Characteristics

Relevant clinical and pathological data were collected from patient's clinical charts (Table 1). All patients and controls were Caucasian. Among 127 tissue samples of bladder carcinoma tested, 100 were from male patients and the remainder from female patients. All normal mucosas ($n = 20$) were collected from males. The majority of cases ($n = 103$) corresponded to primary tumors and only 24 were tumor recurrences. The median age of the individuals with BUCC was significantly higher than those of controls (Mann–Whitney, $P = 0.001$).

Table 1. Clinical and Histopathological Features of Patients With Bladder Urothelial Cell Carcinoma (BUCC) and Normal Bladder Mucosa (NB) Donors

Clinicopathological features	BUCC	NB
Median age, yrs (range)*	72 (35–92)	61 (51–75)
Gender, n (%)		
Male	100 (79)	20 (100)
Female	27 (21)	0 (0)
Histopathological grade, n (%)		
Papillary carcinoma, low grade	48 (38)	n.a.
Papillary carcinoma, high grade	47 (37)	n.a.
Invasive carcinoma	28 (22)	n.a.
Pathological stage, n (%)		
Ta	40 (31)	n.a.
T1	53 (42)	n.a
T2	23 (18)	n.a
T3	3 (2)	n.a
T4	4 (3)	n.a

n.a., not applicable.

Expression Patterns of Class I HDACs in Bladder Tissue and Correlation With the Clinicopathological Parameters

A statistically significant overexpression of all class I HDACs was observed in bladder tumors compared to normal mucosas (Mann–Whitney, $P < 0.0001$ for all, Figure 1A). Protein expression was determined in three randomly selected tumor samples and all displayed higher levels of class I HDACs compared to normal bladder mucosas (Figure 1B), corroborating the results obtained for HDACs transcript levels.

Concerning pathological stage and grade, statistically significant differences were observed only for HDAC1 and HDAC3 ($P < 0.001$, K-W). Pairwise comparisons (NB vs. BUCC; superficial BUCC vs. deeply invasive, and NB vs. low-grade papillary, high-grade papillary or invasive; low-grade papillary vs. high-grade papillary or invasive; high-grade papillary vs. invasive) were also statistically significant (M-W). HDAC1 and HDAC3 were downregulated in deeply invasive and advanced tumors (T2–T4 stages), in comparison to noninvasive and less advanced carcinomas, respectively. Nonetheless, no significant differences were apparent between low-grade papillary and high-grade papillary tumors (Figure 2). Regarding HDAC2 and HDAC8, no differences in transcript levels were found among the different pathological grades and stages. Moreover, no significant association was found between class I HDAC transcript levels and gender (M-W, $P > 0.05$) or age (Spearman's correlation, $P > 0.05$).

Class I HDACs Expression and Patient Survival

Patients with higher levels of HDAC3 had a significantly better disease-specific survival (HR $= 0.40$; 95% CI: 0.17–0.96; **$P = 0.035$), but not DFS, compared to patients with lower transcript levels (Figure 3A). As expected, lower grade and lower pathological stage were also associated with improved DSS (HR $= 8.95$; 95% CI: 3.81–21.01; $P < 0.001$ and HR $= 8.12$; 95% CI: 3.54–18.63; $P < 0.001$, respectively) (Figure 3B and C). Conversely, HDAC1, HDAC2, and HDAC8 did not disclose any prognostic value (either in DSS or DFS) in our dataset. In multivariate analysis, however, no statistically significance was found for any of the molecular variables ($P > 0.05$, for all), although tumor stage disclosed independent prognostic value for DSS ($P = 0.022$), but only in papillary tumors.

Impact of Class I HDACs Silencing on 5637 Cell Line Phenotype

Quantitative RT-PCR for HDAC1, HDAC2, HDAC3, and HDAC8 was performed in several BUCC cell lines (TCCSUP, 5637, T24, and J82). Among all the cell lines
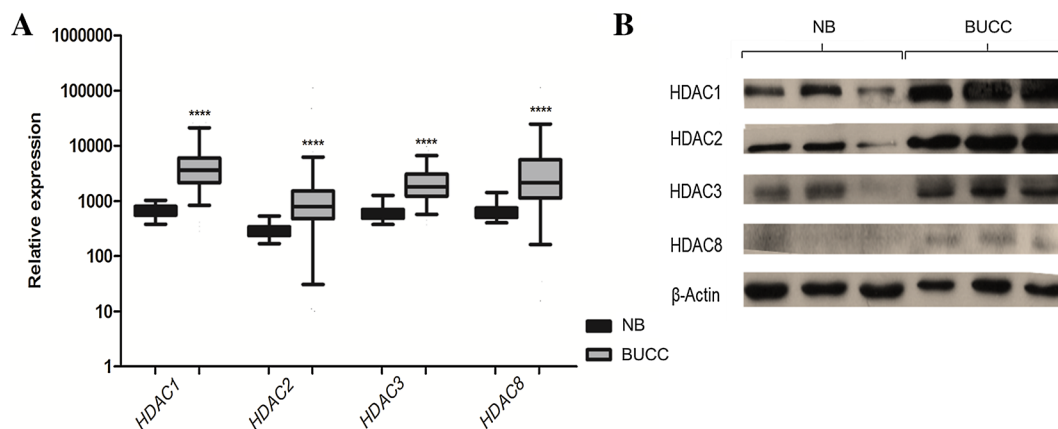

Figure 1. Transcriptional and translational status of class I HDACs expression in BUCC. (A) Distribution of HDAC1, HDAC2, HDAC3, and HDAC8 transcript expression levels in bladder tissues [NB ($n = 20$) and BUCC ($n = 127$)] (****$P < 0.0001$). The represented scale is logarithmic. (B) Protein gel blot analysis of BUCC and NB tissues for class I HDACs. Blots were incubated with a specific antibody recognizing HDAC1, HDAC2, HDAC3, and HDAC8. Their expression was corrected to the constitutive protein, β-actin.
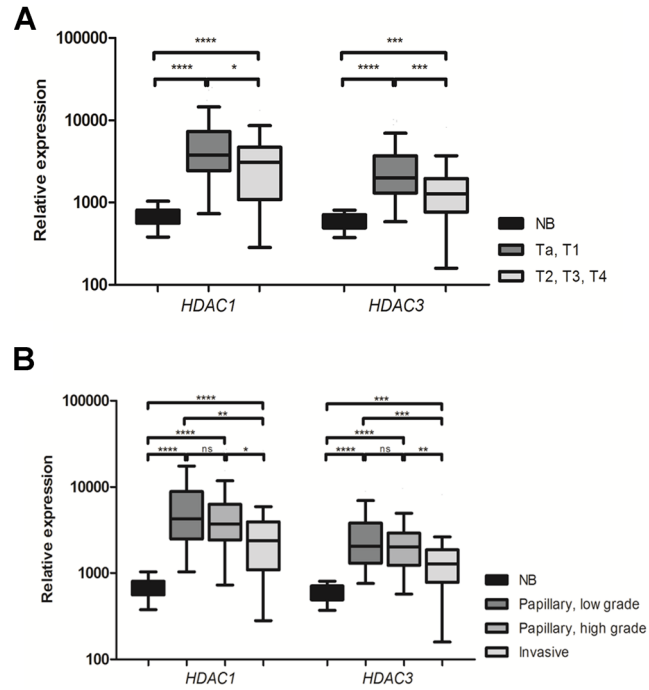
Figure 2. Differential expression of class I HDACs in comparison to clinicopathological variables. (A) Distribution of *HDAC1* and *HDAC3* transcript expression levels in bladder tissues according to their pathological stage. (B) Distribution of *HDAC1* and *HDAC3* transcript expression levels in bladder tissues according to their pathological grade. ****$P < 0.0001$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. The represented scale is logarithmic.
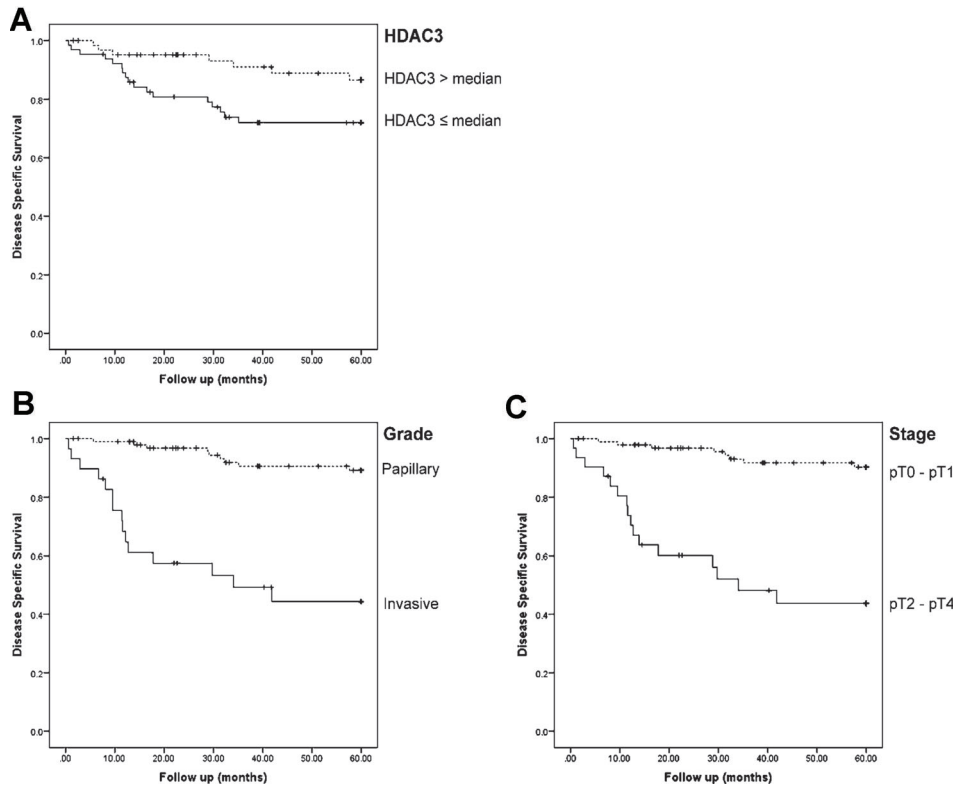

Figure 3. Representative disease-specific survival curves according to HDAC3 expression (A), histopathological grade (B), and tumor stage (C). Differences in survival were calculated using the log-rank test.

analyzed, 5637 showed the higher transcript levels for all HDACs (Supplementary Figure 1) and was, therefore, selected for phenotypic (cell viability, apoptotic, and migration capability) assays following HDAC silencing.

Silencing was successfully accomplished for all class I HDACs, both at mRNA and protein expression levels (Figure 4 and Supplementary Figure 2). An effective reduction in viability was observed following silencing of each class I HDAC (Dunnet's test, $P < 0.001$, Figure 5A). Interestingly, decreased viability was associated with increased levels of p21, a well-known proliferation inhibitor (Supplementary Figure 3). Moreover, significant silencing increased levels of apoptotic cells were apparent at day 3 for all silenced HDACs (Dunnet's test, $P < 0.001$, Figure 5B). Likewise, the number of migrating cells was significantly decreased, but only for HDAC2 and HDAC3-silenced cells (Dunnet's test, $P < 0.001$, Figure 5C). Interestingly, HDAC1 protein levels displayed a significant increase after downregulation of HDAC2 (Figure 4).

DISCUSSION

Increasing evidence has emerged over the last years implicating gene deregulation through the action of HDACs in cancer initiation and progression [24,25]. Recently, emphasis has been placed on the expression of specific HDAC isoforms as a few studies reported aberrant expression of HDAC family members in several tumors, some of which have already been demonstrated to participate in different cell functions, whose deregulation is known to lead to neoplastic transformation [26–31]. Reported data are, however, conflicting because overexpression of a specific HDAC has been associated either with a favorable or a poor prognosis depending on the considered cancer model [31]. Thus, the precise role of class I HDACs deregulation in cancer, and in bladder

carcinogenesis in particular, as well as its putative prognostic value remain elusive.

To determine whether class I HDACs deregulation might be implicated in bladder carcinogenesis, we firstly evaluated HDAC1, HDAC2, HDAC3, and HDAC8 expression in a large set of human BUCC tissues. Overall, mRNA and protein levels of all class I HDACs were upregulated in bladder tumors compared to normal bladder mucosa. These results are in accordance with previous findings in other carcinomas, including colorectal, gastric, and prostatic carcinomas [21–23]. Concerning HDAC1, our results also corroborate a previous report, in which mRNA expression was also found to be increased in a series, although limited, of urothelial tumors ($n = 10$) [32]. These findings strongly support an oncogenic role for class I HDACs in a relatively wide range of common human neoplasms.

Interestingly, *HDAC1* and *HDAC3* expression levels associated, although inversely, with tumor grade and stage. Indeed, less differentiated and more advanced tumors displayed lower expression levels of these HDACs. In other tumor models, however, increased levels of class I HDACs are associated with a more aggressive phenotype [21,23,34]. These apparently contradictory results might be explained based on the two pathways of bladder oncogenesis. In the light of this hypothesis, HDAC1 and HDAC3 overexpression is more pronounced in the superficial urothelial tumors, characterized by *FGFR3*, *HRAS*, and *PI3K* mutations [7,33], which carry the better prognosis, whereas the more clinically aggressive, deeply invasive BUCC display lower expression levels, although still higher than those of normal urothelium. This is further supported by our observation that higher HDAC3 transcript levels significantly associate with longer DSS, although only in univariate analysis. This is in line with previous reports on HDAC3 expression in chronic lymphocytic leukemia [35]. To the best of
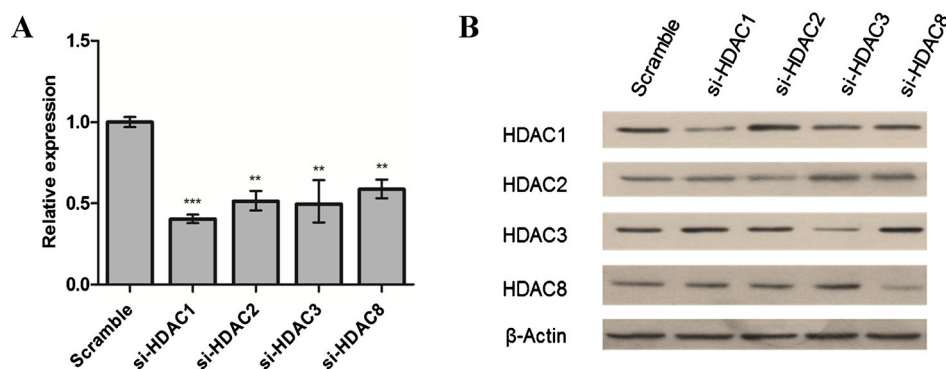

Figure 4. Class I HDACs silencing validation. (A) Relative expression of *HDAC1*, *HDAC2*, *HDAC3*, and *HDAC8* in si-HDAC1, si-HDAC2, si-HDAC3, and si-HDAC8 cells, respectively. Results were normalized to the data obtained with the Scramble (* represent statistically significant differences of si-HDAC1, si-HDAC2, and si-HDAC1&HDAC2, comparing to Scramble: **$P < 0.01$; ***$P < 0.001$). (B) Protein gel blot analysis of 5637 cell line for HDACs silencing. Blots were incubated with a specific antibody recognizing HDAC1, HDAC2, HDAC3, and HDAC8. Their expression was corrected to the constitutive protein, β-actin.
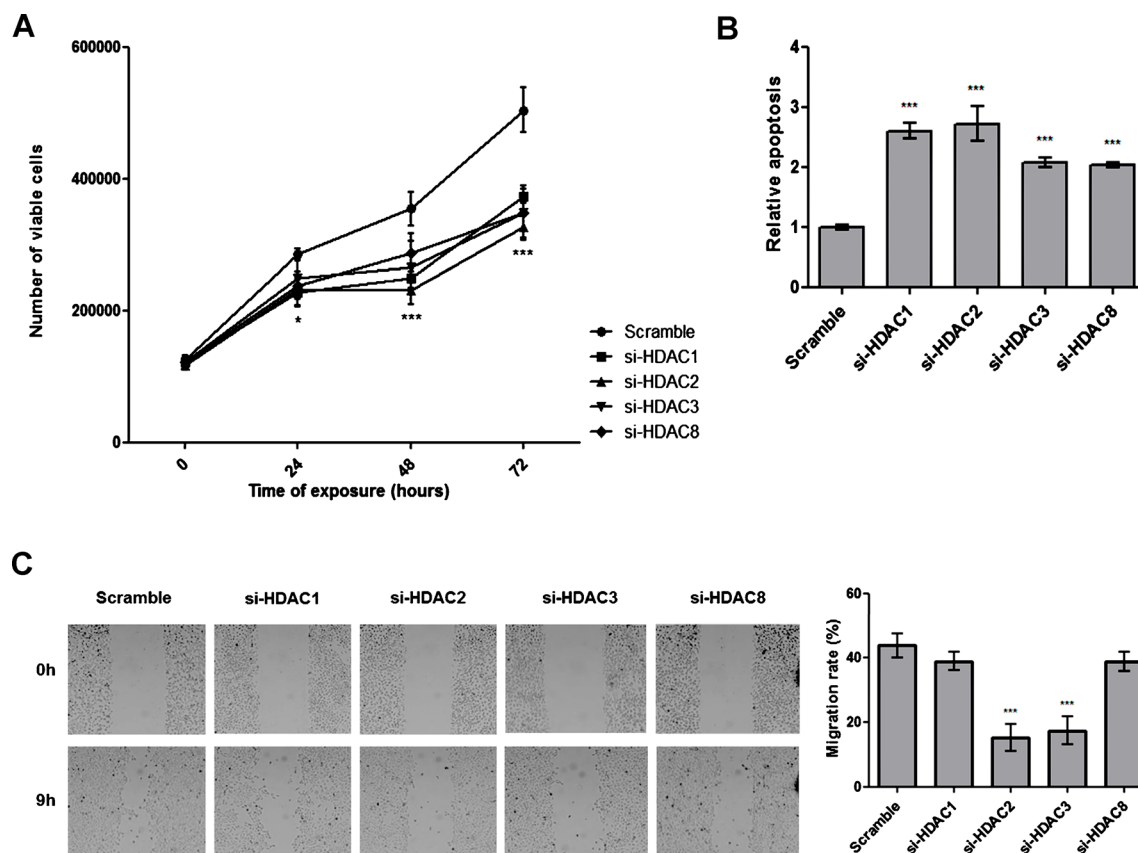
Figure 5. Impact of class I HDACs silencing in the malignant phenotype of 5637 cells. (A) Quantification of cell viability by an MTT assay in Scramble, si-HDAC1, si-HDAC2, si-HDAC3, and HDAC8 cells at 0, 24, 48, and 72 h in culture. (B) Quantification of apoptosis by APOPercentage assay kit of Scramble, si-HDAC1, si-HDAC2 and si-HDAC3 and HDAC8 cells at 72 h in culture. Results were normalized to the data obtained with the Mock. (C) Photographs of the scratch wound assay after the transfection of HDAC1, HDAC2, HDAC3, and HDAC8 siRNA. The migration rate was examined 12 hr after the scratch and only si-HDAC2 and si-HDAC3 significantly reduced the number of migrating cells (* represent statistically significant differences of si-HDACs compared to Scramble: $^*P < 0.05$; $^{***}P \leq 0.001$).

our knowledge, there is no previously published data concerning the prognostic value of HDAC3 expression in BUCC. Hence, our results suggest that class I HDACs' upregulation is a common event in bladder carcinogenesis, occurring in both pathways, although it seems to play a more important role in the initiation and progression of superficial tumors, being less expressive in the deeply invasive tumors. Accordingly, class I HDAC deregulation joins the growing list of epigenetic alterations, like aberrant DNA methylation, which are involved in bladder carcinogenesis [36].

To ascertain the biological role of class I HDACs deregulation, 5637 BUCC cell line was used as an in vitro model, for siRNA experiments. Among the four BUCC cell lines tested, 5637 demonstrated the highest transcript levels of all class I HDACs. Owing to that characteristic and to the fact that this cell line derives from a grade II bladder carcinoma, mimicking superficial BUCC lesions, we believe it represents the ideal model to test the oncogenic role of class I HDACs in BUCC. Silencing was successfully achieved for all HDACs, both at mRNA and protein levels,

allowing us to assess the impact on the malignant phenotype. Remarkably, a significant decrease in cell viability and increased apoptosis was observed for each class I HDACs, which is in line with previous observations in breast [28], colon [22], and lung [37] cancers. Interestingly, several reports suggested that HDAC-mediated repression of genes may cause uncontrolled cell growth, as HDACs repress the transcription of cyclin-dependent kinase inhibitors (CDKIs), such as p21 and p57, allowing for continued proliferation [24,28,38,39]. We were able to confirm this in our study, as a global increase in p21 expression was apparent after class I HDACs silencing. Furthermore, we found that the number of migrating cells was significantly reduced after HDAC2 or HDAC3 silencing. This might be explained by the previously reported role of class I HDACs in the regulation of migration-related genes, such as integrins and MMP2 [40]. Indeed, it has been shown that HDAC3 is involved in altered cell migration in ovarian cancer, although no significant role for HDAC2 has been found in the same model [41]. Globally, these results suggest that overexpression of class I HDACs have an

impact on key cellular pathways influencing the phenotype of malignant urothelial cells. These findings might be of clinical interest because several HDAC inhibitors have been developed and successfully tested as anticancer agents in a wide variety of solid and hematological malignancies.

Interestingly, in the silencing experiments we also observed that HDAC1 protein levels increased after downregulation of HDAC2. However, and in trend with previous publications, the same effect was not observed for HDAC1 mRNA levels [38,42,43]. These findings might be explained by the redundancy and compensatory functions advocated for those two proteins, according to specific knockdown and knockout studies [44]. Thus, increased HDAC1 protein levels may be due to translational or posttranslational changes in HDAC1 occurring in the absence of HDAC2. Intriguingly, a similar effect in HDAC2 (i.e., alterations in mRNA or protein levels) was not observed when HDAC1 was silenced, although HDAC1 and HDAC2 belong to the same co-repressor complex form. Hypothetically, this might be explained by a higher enzymatic efficiency of HDAC2 compared to that of HDAC1. Accordingly, when HDAC1 is downregulated, no vicariation might be required by an increase in HDAC2 expression levels. This hypothesis might also justify the apparently wider impact of HDAC2 silencing in the malignant phenotype, as it impairs migration of BUCC cells, contrarily to HDAC1 silencing.

In conclusion, we showed that all class I HDACs are aberrantly overexpressed in BUCC tissues and in vitro functional assays further suggest an oncogenic role of those HDACs in a human BUCC cell line. Moreover, higher HDAC3 transcript levels are predictive of better outcome in BUCC patients, probably as a result of its association with superficial bladder carcinomas. These results suggest that class I HDACs play an important role in bladder carcinogenesis through deregulation of genes implicated in cell proliferation, cell migration, and apoptosis. Although in vivo experiments were not performed (which might provide additional data to substantiate the observations on primary tissues and cell lines), the alterations depicted in HDACs expression might provide a rationale for future trials investigating the therapeutic usefulness of HDAC inhibitors, already in clinical use for some cancers.

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