Expression of human equilibrative nucleoside transporter 1 (hENT1) and its correlation with gemcitabine uptake and cytotoxicity in mantle cell lymphoma

Background and Objectives. Nucleoside transporters might play a relevant role in the intracellular targeting of many nucleoside analogs used in anticancer therapy. Two gene families (SLC28 and SLC29) encode the two types of human nucleoside transporters, concentrative nucleoside transporter (CNT) and equilibrative nucleoside transporter (ENT) proteins. Chronic lymphocytic leukemia (CLL) cells express both SLC28- and SLC29-related mRNA, although transport function seems to be mostly related to ENT-type transporters. Here we have analyzed the role of nucleoside transporters in nucleoside-derived drug bioavailability and action in mantle cell lymphoma (MCL) cells.

Design and Methods. The relative amounts of hENT1 and hENT2-related mRNA and protein were analyzed in five MCL cell lines and 20 primary MCL tumors by real-time quantitative reverse transcriptase polymerase chain reaction and western blots. Cell viability, measured by annexin V-FITC staining, and nucleoside-derived drug transport were also studied.

Results. MCL cells express higher levels of hENT1 protein than do CLL cells, and a good correlation was found between protein and mRNA levels of hENT1, thus indirectly suggesting that hENT1 might be transcriptionally regulated in MCL cells. More importantly, a significant correlation between these two parameters, drug uptake and sensitivity to gemcitabine, was also observed.

Interpretation and Conclusions. These results further support the concept that nucleoside transporters are implicated in the therapeutic response to nucleoside analogs, and suggest a particular and novel role for hENT1 in the genotoxic response to selected nucleoside analogs, such as gemcitabine, in MCL cells.

Key words: nucleoside transporters, equilibrative nucleoside transporters, gemcitabine, cytotoxicity, mantle cell lymphoma

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cic lymphocytic leukemia (CLL) cells to fludarabine, suggesting a role of the equilibrative nucleoside transporter hENT2 in fludarabine responsiveness in CLL. In contrast to CLL cells, which show defects in the apoptotic machinery, MCL cells are characterized by cell cycle deregulation and additional alterations in cell cycle regulators, particularly in blastoid variants characterized by higher proliferation rates and more aggressive clinical behavior. Since it has previously been suggested that nucleoside transporter proteins play differential roles in cell cycle regulation, we wondered whether nucleoside transporter-dependent cytotoxicity might depend upon different transporter isoforms in particular lymphoproliferative malignancies.

Furthermore, the lack of response of MCL cells to fludarabine in vitro prompted us to analyze the expression of these nucleoside transporters in MCL cells. For this purpose we analyzed the equilibrative transporters in human MCL cell lines (Granta 519, NCEB-1, Rec-1, JVM-2 and Jeko-1) as well as in primary MCL cells. Furthermore the cytotoxic effect of gemcitabine (2,2'-difluorodeoxycytidine), a deoxycytidine analog, and its correlation with drug uptake and with expression of ENT transporters were also analyzed in MCL cells.

**Design and Methods**

**Cell lines**

Five cell lines carrying the t(11;14)(q13;q32) translocation were used: Granta 519, Rec-1, NCEB-1, Jeko-1 and JVM-2. All these cell lines have been described and characterized previously.

**Patients**

Twenty MCL tumors were studied, four of them corresponding to blastoid variant samples (cases #2, #3, #4 and #6). The diagnosis was established according to the World Health Organization classification. The immunophenotype of the tumor was analyzed by immunohistochemistry on tissue sections and/or by flow cytometry on cell suspensions. For cytotoxic studies, cryopreserved cells from eight of these MCL patients were also used. In all these cases, cells were obtained either at diagnosis or relapse, but patients had not been previously treated with nucleoside analogs. In these cases the status of p53 and ATM has been previously analyzed. Informed consent was obtained from each patient in accordance with the Ethical Committee of the Hospital Clinic (Barcelona, Spain).

**Isolation of MCL cells**

Mononuclear cells from peripheral blood samples were isolated on a Ficoll/Hypaque (Seromed, Berlin, Germany) gradient. Tumor cells were obtained after sucluting spleen biopsies with RPMI 1640 culture medium using a fine needle. Cells were used either immediately or after thawing cryopreserved samples. Manipulation due to freezing/thawing did not influence cell response.

**Cell culture**

JVM-2, Rec-1 and NCEB-1 cell lines (0.5x10^6 cells/mL) and tumor cells from patients with MCL (1x10^6 cells/mL) were cultured in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 50 µg/mL penicillin/streptomycin (Gibco, BRL, Paisley, Scotland) and 100 µg/mL noromocin (Amaxa, Khöl, Germany), at 37ºC in a humidified atmosphere containing 5% CO2. The Jeko-1 cell line was incubated in the same conditions but supplemented with 20% FCS. The Granta 519 cell line was cultured at a concentration of 0.5x10^6 cells/mL in DMEM culture medium. Absence of mycoplasma infection was regularly assessed by polymerase chain reaction (PCR) and experiments were performed in mycoplasma-free cells.

**Cell viability assays**

Cell viability were determined simultaneously by double staining with fluorescein isothiocyanate (FITC)-conjugated annexin V (Bender MedSystem, Vienna, Austria) and propidium iodide (PI), as described previously. Cytoxicity was measured as the percentage of annexin V and PI-positive cells. The LD50 was defined as the concentration of drug required to reduce cell viability by 50%.

**Real-time quantitative reverse transcriptase (RT)-PCR**

Total RNA was isolated from each tumor sample and from MCL cell lines using the guanidine thiocyanate method (Ultraspec, Bioket laboratories, Houston, TX, USA). RNA was treated with DNase (Ambion, Austin, TX, USA) to eliminate contaminating DNA. In total, 1 µg of RNA was retrotranscribed to cDNA and the analysis of hENT1, hENT2 and β-glucuronidase (GUS) mRNA was performed by RT-PCR as previously described. The expression of Ki-67 was analyzed using a pre-designed Assay-on-demand (Applied Biosystems, Foster City, CA, USA). The amounts of mRNA were given as arbitrary units using the ACT method (User Bulletin #2, Applied Biosystems) with GUS as an internal control.

**Western blot analysis**

Protein extracts for hENT1 and hENT2 analysis were obtained with 10 mM Tris-Cl (pH 7.4), 0.5% Triton X-100, as described elsewhere. Proteins were separated on 10% sodium dodecylsulfate polyacrylamide gels, transferred to Immobilon-P (Millipore, Bedford, MA. USA) to eliminate contaminating DNA. In total, 1 µg of RNA was retrotranscribed to cDNA and the analysis of hENT1, hENT2 and β-glucuronidase (GUS) mRNA was performed by RT-PCR as previously described. The expression of Ki-67 was analyzed using a pre-designed assay-on-demand (Applied Biosystems, Foster City, CA, USA). The amounts of mRNA were given as arbitrary units using the ACT method (User Bulletin #2, Applied Biosystems) with GUS as an internal control.

**Nucleoside transport**

Nucleoside transport into MCL cell lines was measured using a rapid filtration method, as previously described.


Figure 1. Equilibrative nucleoside transporters (hENT1 and hENT2) in MCL cell lines. Normalized hENT1 and hENT2-related mRNA expression levels in MCL cell lines. CT values for each nucleoside transporter were normalized to an endogenous reference gene (GUS). mRNA expression levels are given in arbitrary units, using JVM-2 as the reference control (A). The experiments were done in duplicate. Western blots of hENT1 and hENT2 and α-tubulin in MCL cell lines. Normalized protein expression levels in these MCL cell lines. Protein amounts were calculated as the densitometric ratio of hENT to α-tubulin, and are shown as arbitrary units (B). Equilibrative nucleoside transport in MCL cell lines. Gemcitabine and uridine transport was measured at 10 seconds and 2 minutes, respectively. NBTI (1 μM) was used to discriminate between NBTI-sensitive (hENT1) and NBTI-insensitive (hENT2) transport rates. Results are given as the means±SE of three to six independent experiments measured in triplicate (C).

was 2 minutes for uridine and 10 seconds for fludarabine and gemcitabine. To discriminate between NBTI-sensitive (hENT1) and NBTI-insensitive (hENT2) transport rates, 1 μM NBTI (Sigma-Aldrich, St. Louis, MO, USA) was used.

Statistical analysis
Correlations between nucleoside transporter expression, nucleoside transport and gemcitabine-induced cytotoxicity were analyzed using the SPSS 11.0 software package (SPSS, Chicago, IL, USA). The significance of correlations was assessed by the Pearson and Mann-Whitney tests.

Results

hENT1 and hENT2 expression in MCL cell lines
The expression of hENT-related mRNA was analyzed by quantitative RT-PCR in human MCL cell lines. Figure 1A shows the amounts of hENT1 and hENT2-related mRNA in five MCL cell lines carrying the t(11;14) (q13;q32). The human JVM-2 cell line was used as a relative calibrator, therefore the expression levels of hENT1 and hENT2 in this cell line were assigned the value of 1 as an arbitrary unit. hENT transporters were expressed in all these cell lines, although a high variability in the levels of expression of hENT1 was observed. Thus, whereas hENT1-related mRNA levels showed a range of variability of nearly 12-fold, hENT2 variability was less than 1-fold. Three cell lines (Granta 519, Jeko-1 and Rec-1) have higher levels of hENT1 compared to the other two cell lines analyzed: NCEB-1 and JVM-2. Amounts of hENT protein were analyzed by western blot in these MCL cell lines using specific antibodies against hENT1 and hENT2 proteins. As recently reported,27 these antibodies specifically recognize single bands of 50-55 kDa. Figure 1B shows western blots of the five cell lines in which hENT1, hENT2 and α-tubulin were analyzed. Semiquantitative analysis of hENT1 and hENT2 expression was achieved by calculating the densitometric ratios of the hENT to α-tubulin, in a range of protein concentrations in which the densitometric signal had been previously shown to be linear. Figure 1C shows the values of these densitometric analyses of the five MCL cell lines. The human JVM-2 cell line was again used as the relative calibrator and its protein amounts assigned the value of 1. The amount of hENT2 protein showed less variability than that of hENT1 protein in MCL cell lines. These results are relatively concordant with the pattern of hENT-related mRNA expression observed using quantitative RT-PCR.

Nucleoside uptake into MCL cell lines
Equilibrative nucleoside transport uptake was analyzed in the five human MCL cell lines. Transport of the natural nucleoside uridine was measured at 2 minutes, as its transport was linear up to 10 minutes (data not shown). Gemcitabine and fludarabine transport rates were measured at 10 seconds, because transport processes were extremely rapid, and thus linear velocity conditions were lost before the first minute of incubation (data not shown). In order to discriminate between hENT1 and hENT2-mediated transport (NBTI-sensitive and -insensitive components, respectively), nucleoside transport was monitored either in the presence or in the absence of 1μM NBTI. Although both hENT1 and hENT2 protein and mRNA were detected in all cell lines, uridine and gemcitabine transport was almost exclusively mediated by the hENT1 transporter (Figure 1C). As was observed in the expression analysis of hENT1, uridine and gemcitabine uptake showed a high range of variability, the Rec-1, Jeko-1 and Granta 519 cell lines having the highest rates for both uridine and gemcitabine uptake. The uptake of gemcitabine mediated by the hENT1 transporter is shown in Table 1. The addition of 1 μM NBTI blocked the gemcitabine uptake, indicating that gemcitabine’s transport is hENT1-mediated (data not shown). Uridine transport significantly correlated with hENT1-related mRNA expression (Figure 2A), determined by quantitative RT-PCR and with hENT1 protein levels (Figure 2B) (p=0.009 and 0.042, respectively). Similar results were obtained for gemcitabine transport (Figure 2C and D) (p=0.086 and p=0.015, respectively), but no correlation was found for fludarabine uptake (data not shown). Furthermore, the amounts of hENT2 protein and hENT2-related mRNA did not correlate with hENT2-mediated uridine and gemcitabine transport rates (data not shown).

Sensitivity of MCL cell lines to gemcitabine
Since in MCL cell lines, the expression levels of hENT1-related mRNA are higher than those of hENT2, we ana-
lyzed the sensitivity of these cells to gemcitabine, a nucleoside analog whose uptake is known to be mediated preferentially by ENT1 transporters. Jeko-1, Rec-1, Granta 519, JVM-2 and NCEB-1 cell lines were incubated for 24 hours with different concentrations of gemcitabine (0.003-50 µg/mL) (Lilly, Hampshire, UK), and the cytotoxic effect was measured by annexin V-FITC/PI staining. The LD<sub>50</sub> for gemcitabine is shown in Table 1. A cytotoxic effect was observed at low doses of gemcitabine (< 3 µg/mL) in the Jeko-1, Granta 519 and Rec-1 cell lines, whereas JVM-2 and NCEB-1 cells were resistant to gemcitabine-induced cytotoxicity. The highest sensitivity was detected in Rec-1 cells (20 ng/mL) and this cell line also had the highest rates of gemcitabine uptake. In accordance with this, the two resistant cell lines (JVM-2 and NCEB-1) had the lowest levels of gemcitabine uptake. These results suggest that lack of response to gemcitabine correlates with low drug uptake rates. In contrast, no direct relationship was observed between gemcitabine cytotoxicity and p53 and ATM status. Thus, no alterations in the DNA damage response genes (p53 and ATM) were detected in Rec-1 cells, although the other two sensitive MCL cell lines carried p53 (Jeko-1) or ATM (Granta 519) alterations. Furthermore, JVM-2 (wild type p53 and ATM) and NCEB-1 (alterations in p53 and ATM) showed no response to gemcitabine.

**Table 1. Cytotoxicity, uptake of gemcitabine and the status of DNA response damage genes in MCL cell lines.**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; Gemcitabine (µg/mL)</th>
<th>Gemcitabine uptake (pmol/mg protein/10 sec)</th>
<th>DNA response damage genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRANTA</td>
<td>2.75±0.37</td>
<td>2.20±0.43</td>
<td>wt del</td>
</tr>
<tr>
<td>JEKO-1</td>
<td>0.28±0.013</td>
<td>2.91±0.31</td>
<td>mut wt</td>
</tr>
<tr>
<td>REC-1</td>
<td>0.02±0.016</td>
<td>4.10±0.52</td>
<td>wt wt</td>
</tr>
<tr>
<td>NCEB-1</td>
<td>NR</td>
<td>0.72±0.19</td>
<td>mut del</td>
</tr>
<tr>
<td>JVM-2</td>
<td>NR</td>
<td>0.44±0.05</td>
<td>wt wt</td>
</tr>
</tbody>
</table>

p53 mutational status assessed by single strand conformational polymorphism analysis (SSCP) and sequencing; ATM status assessed by fluorescent in situ hybridization (FISH); NR: no response.

Correlation of hENT1 protein and mRNA amounts, drug uptake and sensitivity to gemcitabine in MCL cell lines

We did a correlation analysis to assess possible relationships between hENT expression and sensitivity to gemcitabine and the uptake of these drugs into the cells. Sensitivity to gemcitabine (5 µg/mL) was directly correlated with both hENT1-related mRNA expression (Figure 3A) (r=0.9; p=0.04) and hENT1 protein levels (Figure 3B) (r=0.90; p=0.04). Similar results were obtained when cells were incubated with other doses of gemcitabine (data not shown). No correlation between sensitivity to gemcitabine and hENT2 mRNA or protein expression was detected (data not shown). Furthermore, gemcitabine transport also correlated with sensitivity to gemcitabine (r=0.97; p=0.005) in these MCL cell lines (Figure 3C).

**Equilibrative nucleoside transporter expression pattern in MCL cells**

Expression of ENT was analyzed by quantitative RT-PCR in tumor cells from 20 primary MCL tumors. Figure 4A shows the expression of hENT1 and hENT2-related mRNA in the whole cohort of MCL tumors analyzed in this study, using JVM-2 cells as the relative calibrator. The mean values of hENT1 and hENT2-related mRNA in primary chronic lymphocytic leukemia (CLL) cells, described previously, are also included in Figure 4A. The variability in hENT1 and hENT2-related mRNA expression was very
hENT1 correlates with the response to gemcitabine

The expression patterns of these transporters in primary MCL cells differed from those observed previously in CLL cells. Thus, the average expression of hENT1-related mRNA in MCL cells was significantly higher (0.76±0.48) than that reported previously for CLL cells (0.13±0.10) (p<0.001), and hENT2 protein expression was lower (0.89±0.52) compared to that observed in CLL cells (4.04±1.83) (p<0.001). The expression of hENT1 and hENT2 transporters did not correlate with either the morphological MCL variant or the proliferation index of the tumor cells.

Figure 3. Correlation between gemcitabine cytotoxicity and expression of hENT1 and gemcitabine uptake in MCL cell lines. Sensitivity to gemcitabine (5 µg/mL), expressed as the percentage of apoptotic cells after 24 hours of treatment, was plotted against hENT1 mRNA levels (A), hENT1 protein expression (B) and gemcitabine transport into the cells (C). Correlation coefficients and p-values are shown.

Ex vivo sensitivity to gemcitabine in primary cells from MCL patients

Primary cells from eight MCL patients, which had been included in the expression analysis of ENT transporters, were incubated with different concentrations of gemcitabine (0.5-50 µg/mL) for 48 hours. The biological characteristics of these patients and LD₅₀ for gemcitabine are reported in Table 2. In all primary MCL cases analyzed, the doses of gemcitabine necessary to induce a cytotoxic effect were higher than those necessary in sensitive MCL cell lines. In cells from six MCL patients (#13, #14, #15, #17, #18 and #19), the LD₅₀ for gemcitabine were less than 50 µg/mL. In the other two cases (#16 and #20) higher doses of gemcitabine were necessary to achieve a cytotoxic effect. Gemcitabine cytotoxicity did not correlate with p53 and ATM status nor with the proliferation index analyzed by Ki-67 quantification in these primary MCL cells.

Correlation of hENT expression with mRNA expression, and ex vivo gemcitabine cytotoxicity in MCL primary cells

The amounts of hENT1-related mRNA correlated with the hENT1 protein levels (r=0.62; p=0.013) (Figure 5A). As we observed in MCL cell lines, there was a significant correlation between hENT1-related mRNA levels and the cytotoxic effect of gemcitabine at 5 µg/mL (r=0.72; p=0.04) (Figure 5B). Similar results were obtained when primary cells were incubated with other doses of gemcitabine (data not shown). Amounts of hENT2-related mRNA did not correlate with either hENT2 protein level or with cell viability after exposure to different doses of gemcitabine. Furthermore, neither hENT1-related mRNA nor protein expression correlated with the cytotoxic effect induced by other non-nucleoside analog chemotherapeutic agents such as mitoxantrone, a topoisomerase inhibitor (data not shown).

Discussion

CNT and ENT proteins are responsible for the uptake of many nucleoside derivatives used in anticancer therapies. Tumor cells show highly variable patterns of CNT and ENT expression, which might affect drug bioavailability and action. Our previous studies demonstrated that fludarabine accumulation in CLL cells is mostly mediated by ENT-type transporters and we have recently proposed that hENT2, in particular, may play a role in fludarabine responsiveness in CLL patients. MCL-derived cell lines and primary MCL cells express both ENT-type transporters proteins. We have detected higher levels of hENT1 protein and hENT1-related mRNA in MCL cell lines compared to in primary MCL cells and...
CLL cells. In contrast, CLL cells, characterized by the accumulation of B cells in the G0 phase of the cell cycle,25 showed higher expression of the hENT2 isoform than did MCL cells. These results are in agreement with previous reports suggesting a significant role for hENT1 in cell proliferation14,15 and with higher levels of hENT1 expression in immortalized cell lines than in their primary counterparts.26 Furthermore, high levels of hENT1-related mRNA had been previously reported in an isolated case of MCL.27 Heterogeneity of the amounts of hENT1 in human tissues has been described,22,28 particularly in non-Hodgkin's lymphomas, where it has been proposed that expression of hENT1 is linked to B-cell differentiation.29 Moreover, in contrast to the lack of relationship between hENT proteins and mRNA observed in CLL cells,12 a significant correlation was found between the amount of hENT1 protein and corresponding mRNA levels in MCL cells. This correlation supports the view that hENT1 expression might be transcriptionally regulated in this malignancy.

In MCL cell lines, hENT1 expression was significantly correlated with nucleoside transport when uridine and gemcitabine were used as substrates, but not when fludarabine was used. We demonstrated that gemcitabine transport was almost exclusively mediated by the hENT1 transporter. These results might further reflect a different role for hENT1 and hENT2 proteins in gemcitabine and fludarabine-induced cytotoxicities.

Evidence demonstrating that gemcitabine acts as a genotoxic agent in proliferating tumors30,31 along with previous in vitro and in vivo reports showing a lack of responsiveness of MCL cells to fludarabine6,32,33 prompted us to analyze the effect of gemcitabine in MCL cells. Gemcitabine is a deoxycytidine analog that has more effective cellular kinetics, including intracellular incorporation, phosphory-

Table 2. Characteristics of MCL patients and LD50 for gemcitabine.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cell source</th>
<th>Morphological variant</th>
<th>% Tumoral cells</th>
<th>LD50 Gemcitabine (µg/mL)</th>
<th>p53 status</th>
<th>ATM status</th>
<th>Ki67 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>PB</td>
<td>C</td>
<td>95</td>
<td>26.93±0.01</td>
<td>wt</td>
<td>del</td>
<td>26</td>
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<tr>
<td>14</td>
<td>Spleen</td>
<td>C</td>
<td>95</td>
<td>13.36±0.76</td>
<td>wt</td>
<td>del</td>
<td>46</td>
</tr>
<tr>
<td>15</td>
<td>PB</td>
<td>C</td>
<td>84</td>
<td>30.70±0.02</td>
<td>wt</td>
<td>del</td>
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<tr>
<td>16</td>
<td>PB</td>
<td>C</td>
<td>95</td>
<td>58.14±0.01</td>
<td>mut</td>
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</tr>
<tr>
<td>17</td>
<td>Spleen</td>
<td>C</td>
<td>80</td>
<td>5.05±0.01</td>
<td>wt</td>
<td>wt</td>
<td>42</td>
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<tr>
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<td>PB</td>
<td>C</td>
<td>86</td>
<td>8.42±0.01</td>
<td>wt</td>
<td>wt</td>
<td>23</td>
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<tr>
<td>19</td>
<td>PB</td>
<td>C</td>
<td>90</td>
<td>7.72±0.01</td>
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<td>wt</td>
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<tr>
<td>20</td>
<td>PB</td>
<td>C</td>
<td>70</td>
<td>62.56±0.01</td>
<td>wt</td>
<td>wt</td>
<td>13</td>
</tr>
</tbody>
</table>

Source of the cells used for the in vitro analysis. PB: peripheral blood; C: classical variant; p53 status was assessed by FISH, SSCP and sequencing; ATM status was assessed by FISH; Ki67 expression was assessed by RT-PCR. CLL mRNA was used as the relative calibrator and assigned the value of 1 as an arbitrary unit.
Furthermore, gemcitabine is phospho-sensitivated, indicating that resistance to the nucleoside transporters with a number of anti-metabolite drug-analogs have been reported for the NCI-60 panel of cell lines. Although gemcitabine induced a cytotoxic effect in most primary MCL cells, some insensitive cases were also found. Resistance to gemcitabine might involve mechanisms other than transport processes, related to intracellular accumulation, metabolism and targeting.

There is a report of a MCL patient who exhibited resistance to fludarabine with a deficiency in both nucleoside uptake and accumulation, with no major changes in mRNA levels of the genes involved in nucleoside analog uptake and metabolism, indicating that resistance to the nucleoside analogs was downstream of gene transcription or involved other genes. Moreover, MCL cells often show alterations in genes implicated in the DNA damage pathway such as p53 and ATM, which might also explain resistance to nucleoside analogs. In spite of these putative mechanisms of resistance, here it is shown that hENT1 plays an important role in drug cytotoxicity in those primary MCL cells that respond to pharmacological doses of gemcitabine.

In summary, this study demonstrates that MCL cells express high levels of hENT1 compared to CLL cells and that these cells might be more sensitive to nucleoside analogs whose uptake is mediated preferentially by the hENT1 transporter. The results presented in this paper further support the hypothesis that nucleoside transporters are implicated in the therapeutic response to nucleoside analogs, suggesting that levels of hENT1 expression might be useful to predict response to nucleoside analogs known to be taken up via ENT1 carriers, such as gemcitabine in MCL patients. A better understanding of nucleoside analog transport may extend therapeutic strategies and improve the prognosis of MCL patients.

**Figure 5.** Correlations between hENT1 protein and mRNA expression and ex vivo gemcitabine cytotoxicity in primary MCL cells. Correlation between hENT1-related mRNA levels and protein expression in primary MCL cells (A). Correlation of ex vivo sensitivity to gemcitabine with hENT1-related mRNA in gemcitabine-sensitive cases (B). These results are expressed as the percentage of non-viable cells observed after 48 hours treatment with 5 µg/mL gemcitabine. Correlation coefficients and p-values are shown.

SM and MM-A performed all the experiments reported in this paper. They contributed to the interpretation of data and drafting the manuscript. They created the tables and figures of the manuscript. FJ-C participated in the design of the experimental work focused on the nucleoside transporter analysis. NV and EC participated in the design of the experimental work and contributed to the interpretation of data, and the revision of the manuscript. MP-A and DC developed the project, designed the experimental procedures and coordinated them. They contributed to the interpretation of data and drafting the article.

All the authors approved the final version of the manuscript to be published and declare that they have no potential conflict of interest.

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