Low expression of aldehyde dehydrogenase 1 (ALDH1) is a prognostic marker for poor survival in pancreatic cancer

Running title: ALDH-1 is a prognostic marker in pancreatic cancer

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Abstract

Background
Aldehyde dehydrogenase 1 (ALDH1) has been characterized as a cancer stem cell marker in different types of tumors. Besides, it plays a pivotal role for gene regulation and endows tumor cells with augmented chemoresistance. Recently, ALDH1 has been described as a prognostic marker in a pancreatic cancer tissue microarray. The aim of this study was to reevaluate the expression of ALDH1 as a prognostic marker on whole mount tissue sections.

Methods
Real-time-quantitative-PCR (qRT-PCR) and Western Blotting were used to evaluate the expression profile of ALDH1 in eight pancreatic cancer cell lines and one non-malignant pancreatic cell line. Immunostaining against ALDH1 and Ki-67 was performed on paraffin-embedded samples of 97 patients with pancreatic cancer. Immunohistochemical results were correlated to histopathological and clinical data.

Results
As result, qRT-PCR and Western Blotting revealed a different expression pattern of ALDH1 in different malignant and non-malignant pancreatic cell lines. Immunohistochemical analysis demonstrated that ALDH1 was confined to the cellular cytoplasm and occurred in 72 cases (74 %), whereas it was negative in 25 cases (26 %). High expression of ALDH1 was significantly correlated to an increased proliferation rate (Pearson correlation, \( p = 0.045 \)). Univariate and multivariate analyses showed that decreased expression of ALDH1 is an independent adverse prognostic factor for overall survival.

Conclusions
Immunohistochemical analysis on whole mount tissue slides reveals that ALDH1 is more abundantly expressed in pancreatic cancer than initially reported by a tissue microarray analysis. Moreover, high expression of ALDH1 is correlated significantly with the proliferation of tumor cells. Intriguingly, this study is the first which identifies low expression of ALDH1 as an independent adverse prognostic marker for overall survival in pancreatic cancer.

Key words: Pancreatic cancer, ALDH1, prognostic marker, proliferation rate
Introduction

Although the incidence of pancreatic cancer amounts only to 3% of all tumors, it is a major cause of cancer-related death in western countries [1]. Surgical resection remains the only potentially curative therapeutic option. At time of initial diagnosis, only a minority of patients with pancreatic cancer is still in a curable resectable stage [2]. Even if a potentially curative resection can be performed, the 5-year overall survival declines to 10 – 25% [2-4].

Current prognostic markers for curatively resected pancreatic cancer include lymph node status, tumor type and histological grade [2, 4, 5]. However, these prognostic markers only poorly predict metastatic progression or tumor response to medical treatment in the individual patient. Therefore new biomarkers are required in able to stratify patients into different risk categories, thus allowing a more specific treatment regimen.

Stem cell markers are a promising group of new biomarkers. In pancreatic cancer, several surface markers have been identified to provide a subpopulation of the tumor cells with so-called stem cell characteristics. These cancer stem cell markers include CD44 [6], CD24[6] and CD133 [7]. Their relevance as strong prognostic markers in pancreatic cancer has already been evaluated [8, 9].

In this study, we have focused on aldehyde dehydrogenase 1 (ALDH1), which has been recently identified to track tumor stem cells in breast cancer [10], colon cancer [11] lung cancer [12] and head and neck squamous cancer [13]. ALDH1 belongs to the superfamily of NAD(P)(+)-dependent enzymes which metabolize a wide spectrum of endogenous and exogenous aliphatic and aromatic aldehydes [14]. It is distributed ubiquititously in many human tissues where it is localized in the cellular cytoplasm. By the formation of retinoic acid it acts as a pivotal modulator for gene regulation and cell differentiation [14]. Besides, ALDH1 has a strong activity for detoxifying aldophosphamid, hence providing overexpressing cells with chemoresistance against cyclophosphamid [15].

Besides to gynaecological tumors and tumors of the respiratory tract [10, 12], increased expression of ALDH1 in a pancreatic cancer tissue microarray has been recently described to correlate with a dismal prognosis [16]. On the contrary, increased expression of ALDH1 in ovarian cancer correlates with a more favorable disease-free and overall survival [17]. The
aim of our study was to reevaluate the expression pattern of ALDH1 in pancreatic cancer on whole-mount tissue slides and to correlate these results with clinical and pathological data. Furthermore, ALDH1-positive non-pancreatic tumor cells have been found to have a high Ki-67 expression [12, 18, 19]. Therefore, we focused on the correlation between the expression of ALDH1 and the proliferation rate of the pancreatic cancer samples. Our study demonstrates that high expression of ALDH1 is correlated significantly with the proliferation rate of pancreatic tumor cells. Intriguingly, this study is the first which identifies low expression of ALDH1 as an independent adverse prognostic marker for overall survival in pancreatic cancer.

Materials and methods

Patients
Paraffin-embedded samples of primary pancreatic ductal adenocarcinoma from a consecutive series of 97 patients, who underwent tumor resection between 2002 and 2005 at the Department of General, Visceral, and Transplantation Surgery, University of Heidelberg, were included in this study. Previously, we had evaluated the prognostic significance of CD166 in this patient cohort [20]. No neoadjuvant radio- or chemotherapy was applied prior to surgical resection to any patient. After resection, 70 patients were subjected to adjuvant chemotherapy. The median observation period for overall survival was 18.3 months. Paraffin-embedded tumor samples were provided from the tissue bank of the National Center for tumor disease (NCT) Heidelberg. The tissue sampling and the analyses regarding potential prognostic markers were approved by the local ethics committee. Table 1 displays the clinical and histopathological characteristics of the patients.

Immunohistochemistry
Immunohistochemical staining for ALDH1 was performed as described previously [20]. Briefly, 1 - 2 µm sections of formalin-fixed, paraffin-embedded tumor samples were mounted on object slides (SUPERFROST® PLUS microscope slides, Menzel, Braunschweig, Germany) and incubated at 37°C over night. After dewaxing in xylene and ethanol, antigen
retrieval was achieved by boiling in a microwave oven for 5 min (pH 6.0, 0.94 ml Antigen Unmasking Solution, Vector Laboratories, Burlingame, CA, USA) / 100 ml distilled water) for three times. After immersing slides in a 3.0 % hydrogen peroxidase solution in methanol for 20 min in order to inhibit endogenous peroxidase activity, nonspecific binding sites were blocked by preincubation with 10 % normal goat serum (Vector Laboratories) in 1 mol/L PBS for 30 min at room temperature. Primary antibodies against ALDH1 (Mouse IgG1, clone 44, BD Transduction Laboratories™, Europe, dilution 1:200, Rabbit IgG, clone EP1933Y, Novus Biologicals, USA, 1:200, Rabbit polyclonal, Novus Biologicals, USA 1:50) and IgG – negative control (Mouse IgG, BD Pharmingen, Europe, 1:100) were incubated at 4 °C over night. Slides were loaded with secondary antibody coupled with peroxidase-conjugated polymers (EnVision®+ System,DakoCytomation A/S, Denmark) for 30 minutes. Afterwards, immunoreaction was visualized by using AEC Substrate Chromogen (DakoCytomation A/S) according to the instructions of the manufacturer. Eventually, sections were counterstained with haematoxylin, dehydrated in graded concentrations of ethanol, and mounted.

Staining against Ki-67 was performed at the tissue bank of the National Center for tumor disease (NCT) Heidelberg by the mean of an automated immunostainer (Dako Autostainer, A/S, Denmark) according to the manufacturer's instructions. Briefly, paraffin was removed with xylene and ethanol before the sections were demasked 15 min (pH 6.0, 0.94 ml Antigen Unmasking Solution, Vector Laboratories, Burlingame, CA, USA) / 100 ml distilled water). The slides were loaded with the primary antibody (Ki-67, Dako, A/S, Denmark 1:200) and with the secondary antibody (Dako REAL Biotinylated Secondary Antibodies, A/S, Denmark) for 30 minutes, respectively. As detection system, a streptavidin-peroxidase complex (Dako REAL Detection System) was used and the reactions were visualized by using Dako REAL AEC/H2O2 Substrate Solution (CHROM). Finally, the slides were counterstained with haematoxylin.

Estimated percentage of positive cells for ALDH1 was evaluated for each slide by three independent researchers (CK, JB, NM) and two pathologists (CM, FB) on a blind basis. The percentage of positive cells was categorised as 0: (< 5 %), 1 ( 5 – 25 %), 2 ( > 25 % - 50 %), 3 (> 50 % - 75 %), and 4 ( > 75 %). Likewise, the proliferation rate of each tumor specimen
was determined on a blind basis by estimating the percentage of tumor cells stained positive against Ki-67.

Cell lines
Following cell lines were used: SU8686, BxPC3, PANC-1, AsPC-1, MiaPaCa-2, ACBRI (all purchased from The American Type Culture Collection (ATCC), Manassas, VA 20108, USA), Colo357 (provided by T. Welsch) and T3M-4 (Cell Bank, RIKEN BioResource Center, Japan). In June 2010, the cell lines were retested and reauthenticated by analyzing cell samples for eight polymorphic short random repeats (STRs) loci (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany). Tumor cell lines were maintained in RPMI-1640 (Sigma, St. Louis, MO), supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin. ACBRI cells were maintained in CSC Certified Complete Medium (Cell systems). All cells were cultured in a humidified atmosphere of 5% CO2 at 37°C.

Total-RNA isolation and real-time qPCR
Total-RNA from cell lines was extracted employing RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manual’s instructions. 1 µg Total-RNA was reverse transcribed using the miScript Reverse Transcription Kit (Qiagen, Hilden, Germany). 10 ng of the resulting cDNA was used for quantification by qPCR (SYBR Green PCR Kit, Qiagen, Hilden, Germany) in a Roche Light Cycler™ (Roche Diagnostics GmbH, Mannheim, Germany). Ready primer pairs specific for the genes of ALDH1 and 18S were purchased from Qiagen (Hs_ALDH1A1_1_SG QuantiTect Primer Assay, Cat. No. QT00013286 and Hs_RRN18S_1_SG QuantiTect Primer Assay, Cat. No. QT00199367). All experiments were performed as triplicates. ALDH1 expression was normalized to 18s and quantified as described [21].

Western blotting
Cells were separated with 2 ml Accutase (PAA Cell Culture Company), washed with PBS and lysed by a cell lysis buffer (RIPA) with a protease inhibitor (Complete Mini, Roche Applied Science, Mannheim, Germany). Subsequently, they were disrupted by sonication and the protein concentration of the supernatant (centrifugation: 10 min, 11,000 rpm, 4°C) was determined by a BCA-Kit (Thermo scientific, Schwerte, Germany). 30 µg of protein were separated on 10% SDS-PAGE, transferred to a nitrocellulose membrane (BioRad, Munich, Germany) and incubated with antibodies against ALDH-1 (Mouse IgG1, clone 44, BD Transduction Laboratories™) and β-actin (Sigma-Aldrich, Munich, Germany) with a dilution of 1:500. The horseradish peroxidase-conjugated secondary antibody anti-mouse (Sigma-Aldrich, Munich, Germany) was added at a dilution of 1:2000 after repeated washing with a wash/blocking buffer (PBS, Tween20 0.1% (v/v), skim milk powder 7.5% (w/v)). The antigen-antibody complexes were detected by using an ECL kit (Thermo scientific, Schwerte, Germany) according to the manufacturer’s recommendations. All experiments were performed as triplicates.

Statistical analysis

The software package SPSS, version 11.0 (SPSS, Chicago, IL, USA) was used for all calculations. Chi square ($\chi^2$) test was applied to examine correlation with ALDH1 expression and clinical and pathological parameters. Pearson correlation was employed for determining a correlation between the expression of ALDH-1 and Ki-67. The Kaplan-Meier method was used to visualize overall survival curves, and differences between survival curves were assessed with the log-rank test for univariate analysis.

The Cox proportional hazards regression model was conducted on all covariates that showed significant association with overall survival in univariate analysis. P-values of all statistical tests were two-sided and $\rho < 0.05$ was considered significant.

Results

Evaluation of specificity of ALDH1 immunohistochemistry

To validate the specificity of immunohistochemical staining against ALDH1, we repeated immunohistochemistry in 10 representative specimen with three different antibodies against
ALDH1 and one IgG – negative control. When comparing the immunohistochemical results of the three antibodies in sequential sections, they displayed similar cytoplasmic expression patterns in pancreatic cancer and non-malignant cells, though the staining intensity of the polyclonal antibody was weaker than the staining intensity of the monoclonal antibodies (data not shown). These results, in addition to the Western blotting analysis, confirm the specificity of the anti-ALDH1 staining. For immunohistochemical evaluation of all clinical specimen, we used only the mouse monoclonal antibody (Mouse IgG1, clone 44, BD Transduction Laboratories™, Europe), which had been successfully approved before [10, 12, 17, 18].

Expression of ALDH1 in pancreatic cell lines

In 8 pancreatic cancer cell lines and 1 primary pancreatic epithelial cell line, we assessed the expression of ALDH1 by real-time qPCR and western blotting (Figure 1 A and B). This analysis showed a various abundance of ALDH1 in different pancreatic cell lines. By qPCR, ALDH1 was below the detection threshold in BxPC3, T3M4 and PANC1. In SU8686 and Colo-357, gene transcripts of ALDH1 were down regulated by a 0.07 - and 0.5 – fold change respectively, compared to the non-malignant epithelial pancreatic cell line. In AsPC-1 ALDH1 was increased by 9-fold and in MiaPaCa-2 by 3-fold compared to ACBRI cells. These results were in good line with the western blotting analysis (Figure 1B). In summary, these data may be helpful for further in vitro studies when assessing the relevance of ALDH1 in pancreatic cancer.

Localization of ALDH1 in normal pancreatic tissue and pancreatic cancer cells

In non-malignant pancreatic tissue, adjacent to pancreatic cancer in 60 cases, we observed a strong cytoplasmic expression of ALDH1 in the pancreatic islet cells, in the acinar epithelium and in the ductal epithelial cells. The staining intensity displayed a homogenous pattern: islet cells usually revealed a strong staining intensity, acinar and ductal epithelial cells revealed a moderate staining intensity.

In pancreatic cancer, the expression of ALDH1 was confined to the cellular cytoplasm and occurred in 72 cases (74 %), whereas it was negative in 25 cases (26 %) (Figure 2 A – D).
The percentage of positive cells was categorized as follows: < 5 %: 25 samples (26 %), 5 – 25 %: 18 samples (18 %), 26 - 50 %: 16 samples (17 %), 51 - 75 %: 18 samples (18 %), > 75 %: 20 samples (21 %). As already previously reported in a small cohort of patient samples [22], high expression of ALDH1 was more often observed in well differentiated tumors, though the correlation between expression and tumor grades did not reach statistical significance (Table 1).

**Relationship between expression of ALDH1 and Ki-67**

Recently, ALDH1 positive tumors have been found to have a high Ki-67 expression and to correlate positively with an increased proliferative capacity in vitro [12, 18, 19]. Therefore, we performed immunostaining against Ki-67 in consecutive sections and compared the results with the expression of ALDH1 in pancreatic cancer (Figures 2 E – H). The median proliferation rate reached 15 % (mean: 19 %, range: 1 – 90 %). The correlation between the proliferation rate and the immunohistochemical score of ALDH1 expression was shown to be significant (p=0.045).

**Low expression of ALDH1 is an independent prognostic marker in pancreatic cancer**

To compare the expression of ALDH1 with clinical and pathological parameters, samples were grouped as ALDH1\textsuperscript{negative/low} (percentage of positive cells ≤ median percentage of positive cells) and as ALDH1\textsuperscript{high} (percentage of positive cells > median percentage of positive cells). Chi square test revealed no significant correlation of ALDH1 expression to age, gender, lymph node status, grading, American Joint Cancer Committee (AJCC) tumor stage, or resection status (Table 1).

By univariate analysis using the log-rank test, ALDH1\textsuperscript{negative/low} tumors displayed a significant reduced median overall survival than ALDH1\textsuperscript{high} tumors (ALDH1\textsuperscript{negative/low}: median overall survival 16.3 months, ALDH1\textsuperscript{high}: median overall survival 33.0 months, p = 0.003) (Figure 3A). Furthermore, univariate analysis by log rank test demonstrated lymph node status, AJCC tumor stage and treatment with adjuvant chemotherapy to be significant prognostic parameters as described recently [20] (Table 2). Univariate analysis for expression of Ki-67,
performed by categorizing the tumor samples into Ki-67\textsuperscript{low} (proliferation rate $\leq$ median proliferation rate) and Ki-67\textsuperscript{high} (proliferation rate $>$ median proliferation rate) showed no significant difference for overall survival (Ki-67\textsuperscript{low}: median overall survival 21.8 months, Ki-67\textsuperscript{high}: median overall survival 17.7 months, $p = 0.27$). These results are in good accordance with previous observations [23, 24].

Multivariate analysis with the Cox proportional hazards regression model was performed to identify independent prognostic markers for overall survival. Low expression of ALDH1 was found to be an independent prognostic marker for overall survival ($p = 0.002$) in addition to AJCC tumor stage ($p = 0.002$) and treatment with adjuvant chemotherapy ($p = 0.0001$) (Table 3a). Lymph node status was not included into the multivariate analysis due to its linear depending covariance with AJCC stage.

**Subgroup analysis of expression of ALDH1**

ALDH1 provides tumor cells with increased resistance against chemotherapeutical agents such as cyclophosphamid [15]. However, there exist also data that increased expression of ALDH1 is associated with an improved response to chemotherapy in ovarian cancer [17]. For that reason, we performed an univariate analysis for the subgroup of patients having received adjuvant chemotherapy ($n = 69$) and for the subgroup of patients having not received adjuvant treatment ($n = 19$). In this analysis, we identified strong expression of ALDH1 as a significant prognostic marker for a more favorable overall survival in the subgroup of patients having been treated with chemotherapy adjuvantly ($p = 0.012$). (Figure 3B).

A multivariate analysis including AJCC stage confirmed ALDH1\textsuperscript{high} in the subgroup of patients having received adjuvant chemotherapy as a significant prognostic marker ($p = 0.01$) in addition to AJCC stage ($p = 0.004$). Univariate analysis with adjuvant chemotherapy was excluded for its linear depending covariance (Table 3b).

In the smaller subgroup of patients having not received chemotherapy postoperatively in adjuvant intention ($n = 18$), different expression of ALDH1 had no significant impact as prognostic marker on overall survival ($p = 0.43$) by univariate analysis.
Discussion

In our study, we have demonstrated that low expression of ALDH1 is an independent prognostic marker for shortened disease-free and overall survival in ductal adenocarcinoma of the pancreas. These results are very conflicting to a recent published study by Rasheed et al., who have described increased expression of ALDH1 in pancreatic cancer to correlate with a dismal prognosis [16]. Since Rasheed et al. have evaluated the expression of ALDH1 by tissue microarrays, whereas we performed our immunohistochemical analysis on whole mount tissue slides, methodological differences may explain these opposing results. However, by evaluating whole mount tissue slides, we found ALDH1 to be expressed very heterogeneously within the tumor bulk. In fact, while Rasheed et al. claimed only 34% of the immunostained tumor samples as positive, we observed a much higher fraction of 74% of tumor specimen to be positive. Hence, using only 0.6 mm tissue microarray samples may result in an increased rate of false-negative results.

However, akin to the observations by Morimoto et al. in breast cancer [18] and by Jiang et al. in lung cancer cell lines [12], we have found a positive correlation between an increased expression of ALDH1 and a higher proliferation rate. Furthermore, we observed strong expression of ALDH1 more frequently in well differentiated tumor samples. These findings are tempting to hypothesize, why overexpression of ALDH1 correlates to a more favorable clinical outcome: assuming that conventional cytotoxic therapy aims mainly at the proliferating, differentiated cellular fraction, it can be conjectured that overexpression of ALDH1 is associated with a biological type of tumor which is more prone to chemotherapy. This hypothesis is supported by our findings, that increased expression of ALDH1 has been identified as a strong prognostic marker for improved clinical outcome in our subgroup analysis for patients having received adjuvant chemotherapy but not in the subgroup of patients having not received adjuvant treatment. Due to the low patient number in this subgroup of patients, however, this needs to be confirmed in further studies. In addition, our assumptions are in line with the clinical results of Chang et al., who reported about a significant correlation between high expression of ALDH1 in ovarian cancer and response to
chemotherapy [17]. Bearing in mind that ALDH1 increases chemoresistance against cyclophosphamid, one can argue that this chemotherapeutical agent is not part of the standard chemotherapy regimen against pancreatic cancer. Moreover, increased chemoresistance against cyclophosphamid by ALDH1 does not exclude increased chemosensitivity to other chemotherapeutic agents. Regarding our in vitro data, we have shown that MiaPaca2 cells have a strong expression of ALDH1. Intriguingly, Monti et al. have demonstrated that MiaPaca2 cells exhibited the strongest response to chemotherapy when exposed to a combined treatment with gemcitabine and 5-FU [25]. These data would favor the hypothesis, that increased expression of ALDH1 is not necessarily related to increased general chemoresistance but depends on the chemotherapeutical agent.

**Conclusion**

In summary, our study describes low expression of ALDH1 as an independent prognostic marker for a poor clinical outcome in pancreatic cancer on whole mount tissue slides. These data are conflicting with a previous report, which has claimed increased expression of ALDH1 to be an adverse prognostic marker in a retrospective study. Therefore, to evaluate the role of ALDH1 as a prognostic and predictive marker for tumor progression and response to chemotherapy in pancreatic cancer, standardized prospective studies with a larger number of patients are required.

**Disclosure of potential competing interest**

No potential conflicts of interest were disclosed.

**Authors’ contribution**

CK designed the study and drafted the manuscript, FB, JB, CK, CM evaluated the immunostaining and followed the patients, EH provided the clinical specimens for this study, CK, JB, TW, TN and SD performed the research and analyzed the data, MK and JW supervised research, analyzed the data and edited the paper. All authors read and approved the final manuscript.
Acknowledgment

We thank Bettina Walter from the tissue bank of the National Center for tumor disease (NCT) Heidelberg for technical help in tissue sections and immunhistology.
References


**Figure 1:** (A) Gene expression analysis of ALDH1 by qPCR in 7 pancreatic cancer cell lines and 1 non-malignant pancreatic cell line. Fold-change (y – axis) of ALDH1 expression in pancreatic cancer cell lines compared to the non-malignant pancreatic epithelial cell ACBRI (7), respectively. (B) Western blotting analysis of ALDH1 in 7 pancreatic cancer cell lines and 1 non-malignant pancreatic cell line (7). MW 55 kD: ALDH1. MW 40 kD: β-actin as loading control. 1: BxPC3, 2: T3M4, 3: MiaPaCa-2, 4: AsPC-1, 5: Colo357, 6: Panc1, 7: ACBRI, 8: SU8686.

**Figure 2:** Immunohistochemical analyses of ALDH1 in pancreatic cancer revealed a cytoplasmic expression varying from 0 – 3. Comparing expression of ALDH1 and Ki-67 in corresponding sequential slides resulted in a positive correlation between strong expression of ALDH1 and a high proliferation rate. (A – D): pancreatic tumor samples displaying cytoplasmic expression of 0 (A), 1 (B), 2 (C) and 3 (D). (E – H): corresponding sequential slides with immunohistochemical staining against Ki-67. Original magnification x 200.

**Figure 3:** (A) Kaplan – Meier curves displaying median overall survival in 97 patients with pancreatic cancer and ALDH1\(_{\text{negative/low}}\) or ALDH1\(_{\text{high}}\) expression. (log-rank test, \(p = 0.003\)) (B) Kaplan – Meier curves displaying median overall survival in a subgroup analyses of patients having received adjuvant chemotherapy. (log-rank test, \(p = 0.012\))
Table 1 Correlation between expression of ALDH1 in pancreatic cancer and clinical and pathological parameters

<table>
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ALDH\textsubscript{1}\textsuperscript{negative/low}: percentage of positive cells ≤ median percentage of positive cells; ALDH\textsubscript{1}\textsuperscript{high}: percentage of positive cells > median percentage of positive cells. P-Value evaluated by the Chi square ($\chi^2$) test.
Table 2 Univariate analysis for prognostic parameters in pancreatic cancer for median overall survival (OAS).

<table>
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(† type of adjuvant treatment in 9 cases unknown)
Table 3 (A) Multivariate analysis (Cox regression model) of prognostic parameters for overall survival in pancreatic cancer

<table>
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<th>Characteristics</th>
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<th>Standard-Error</th>
<th>Wald</th>
<th>Df</th>
<th>Relative Risk</th>
<th>95% CI of Relative Risk</th>
<th>P-Value</th>
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<td>0.293</td>
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<td>1</td>
<td>0.397</td>
<td>0.224–0.705</td>
<td>0.002</td>
</tr>
<tr>
<td>AJCC stage</td>
<td>0.891</td>
<td>0.288</td>
<td>9.592</td>
<td>1</td>
<td>2.438</td>
<td>1.387–4.286</td>
<td>0.002</td>
</tr>
<tr>
<td>Adjuvant Chemotherapy vs. no adjuvant Chemotherapy</td>
<td>-1.813</td>
<td>0.311</td>
<td>33.916</td>
<td>1</td>
<td>0.163</td>
<td>0.089–0.300</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 3 (B) Multivariate analysis (Cox regression model) of prognostic parameters for overall survival in pancreatic cancer in the subgroup of patients having received adjuvant chemotherapy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Beta</th>
<th>Standard-Error</th>
<th>Wald</th>
<th>Df</th>
<th>Relative Risk</th>
<th>95% CI of Relative Risk</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH-1 expression negative/low vs. high</td>
<td>-.804</td>
<td>0.328</td>
<td>5.995</td>
<td>1</td>
<td>0.448</td>
<td>0.235–0.852</td>
<td>0.012</td>
</tr>
<tr>
<td>AJCC stage</td>
<td>-0.804</td>
<td>0.328</td>
<td>8.455</td>
<td>1</td>
<td>2.658</td>
<td>1.375–5.138</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Figure 1

(A) Bar graph showing fold change for various pancreatic cell lines. The x-axis represents the cell lines, and the y-axis shows the fold change.

(B) Western blot analysis with bands for ALDH-1 (55 kD) and β-Actin (40 kD).
Figure 3B

Cumulative Overall Survival

Overall Survival (months)

ALDH1
- High
- Low

- Censored