CrossMark

ALDH-1 expression levels predict response or resistance to preoperative chemoradiation in resectable esophageal cancer patients

J.A. Ajani[a,*], X. Wang[b], S. Song[a], A. Suzuki[a], T. Taketa[a], K. Sudo[a], R. Wadhwa[a], W.L. Hofstetter[c], R. Komaki[d], D.M. Maru[e], J.H. Lee[f], M.S. Bhutani[f], B. Weston[f], V. Baladandayuthapani[b], Y. Yao[a], S. Honjo[a], A.W. Scott[a], H.D. Skinner[d], R.L. Johnson[g], D. Berry[b]

[a]Department of Gastrointestinal Medical Oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[b]Department of Biostatistics, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[c]Department of Cardiac and Thoracic Surgery, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[d]Department of Radiation Oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[e]Department of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[f]Department of Gastroenterology, Hepatology, and Nutrition, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA
[g]Department of Genetics, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston 77030, USA

ARTICLE INFO

ABSTRACT

Purpose: Operable thoracic esophageal/gastroesophageal junction carcinoma (EC) is often treated with chemoradiation and surgery but tumor responses are unpredictable and heterogeneous. We hypothesized that aldehyde dehydrogenase-1 (*ALDH-1*) could be associated with response.

Methods: The labeling indices (LIs) of ALDH-1 by immunohistochemistry in untreated tumor specimens were established in EC patients who had chemoradiation and surgery. Univariate logistic regression and 3-fold cross validation were carried out for the training (67% of patients) and validation (33%) sets. Non-clinical experiments in EC cells were performed to generate complimentary data.

Results: Of 167 EC patients analyzed, 40 (24%) had a pathologic complete response (pathCR) and 27 (16%) had an extremely resistant (exCRTR) cancer. The median ALDH-1 LI was 0.2 (range, 0.01–0.85). There was a significant association between pathCR and low ALDH-1 LI ($p \leq 0.001$; odds-ratio [OR] = 0.432). The 3-fold cross validation led to a concordance index (C-index) of 0.798 for the fitted model. There was a significant association between exCRTR and high ALDH-1 LI ($p \leq 0.001$; OR = 3.782). The 3-fold cross validation led to the C-index of 0.960 for the fitted model. In several cell lines, higher ALDH-1 LIs correlated with resistant/

---

* *Corresponding author.* Tel.: +1 713 792 2828; fax: +1 713 745 1163.
E-mail addresses: jajani@mdanderson.org, jafferajani@mac.com (J.A. Ajani).

aggressive phenotype. Cells with induced chemotherapy resistance upregulated ALDH-1 and resistance conferring genes (*SOX9* and *YAP1*). Sorted ALDH-1+ cells were more resistant and had an aggressive phenotype in tumor spheres than ALDH-1− cells.

Conclusions: Our clinical and non-clinical data demonstrate that ALDH-1 LIs are predictive of response to therapy and further research could lead to individualized therapeutic strategies and novel therapeutic targets for EC patients.

1. Introduction

Esophageal cancer (EC) remains a global health problem with more than 600,000 new cases diagnosed each year (Jemal et al., 2011). While the incidence of squamous cell histology has been declining, that of adenocarcinoma of the esophagus and or gastroesophageal junction has been rising at an annual rate of 1.5% since 1998 in the USA (Thrift and Whiteman, 2012). Approximately 50% of newly diagnosed patients have local-regional cancer. When feasible, based on the extent of the tumor and co-morbidities, patients with localized thoracic EC are often treated with chemoradiation then surgery (trimodality therapy; TMT), (van Hagen et al., 2012; Ajani et al., 2011) because primary surgery (Rice et al., 2009; Kelsen et al., 1998) or preoperative chemotherapy produces lower cure rates (Allum et al., 2009).

One distinct issue with preoperative therapy is that of unpredictability of response to therapy and patient outcome. Approximately 20% of patients achieve a pathologic complete response (defined as no residual cancer cells in the resected specimen; pathCR). Patients with pathCR often have a longer survival (Rizk et al., 2007; Rohatgi et al., 2005a) and have a lower rate of distant relapse than those who have <pathCR (Rohatgi et al., 2005b). In ∼20% of cases tumor is extremely resistant (defined as ≥50% residual cancer in the surgical specimen; exCRTR) (Chirieac et al., 2005). One challenge is to predict the outcomes prior to surgery (spare surgery in some cases with pathCR) and another is to predict the possibility of exCRTR prior to chemoradiation (avoid chemoradiation in some, if possible). We have reported a clinical variables model that is associated with pathCR but the specificity and sensitivity of the model was low for clinical implementation (Ajani et al., 2012). Additionally, clinical response after chemoradiation is not associated with pathCR (Cheedella et al., 2012). There is not an established biomarker(s) model for clinical implementation.

We focused on aldehyde dehydrogenase-1 (*ALDH-1*) as a potential biomarker because our prior data suggested that the cancer stem cell (CSC) markers participate in repopulation of resistant EC (Sims-Mourtada et al., 2006, 2007). CSCs are a chemotherapy-resistant population capable of self-renewal (Awad et al., 2010). *ALDH-1* is a marker of normal and malignant human mammary stem cells and a prognosticator of poor clinical outcome (Ginestier et al., 2007). ALDH-1 is highly expressed in the tumorigenic cell population of various cancers including lung, breast, (Ricardo et al., 2011) ovarian, (Wang et al., 2012a) pancreas, (Kim et al., 2011) brain, colon, and head and neck (Nguyen et al., 2011). Jiang et al. reported that isolated NSCLC cells with over-expression of ALDH-1

(and CD133) have *in vitro* features of CSCs, including proliferation, self-renewal, differentiation, and resistance to chemotherapy (Jiang et al., 2009; Moreb et al., 2008). Similarly, ALDH-1 over-expressing ovarian cancer cells that were enriched by CD44 were resistant to chemotherapy (Wang et al., 2012a). In breast carcinomas, high ALDH-1 activity identified tumorigenic cell fraction that could recapitulate the heterogeneity of the parental tumor (Ginestier et al., 2007; Nogami et al., 2012). The association of ALDH-1 with chemotherapy-resistance has been reported in mantle cell lymphoma (Brennan et al., 2010) and pancreatic adenocarcinoma (Kim et al., 2011). In EC, ALDH-1 has been associated with nodal metastases and poor prognosis (Wang et al., 2012b; Zhang et al., 2012).

Our analyses are unique in that we report substantial response to therapy data in a large cohort of EC patient population treated with TMT but most importantly, we report the predictive value of ALDH-1 that has not been reported previously and considerable complementary non-clinical experimental results in EC cells that have not been reported previously.

2. Patients and methods

2.1. Patient population

Eligible patients had to have a histologic documentation of the adenocarcinoma or squamous cell carcinoma of the thoracic EC. In addition, patients were required to have complete clinical staging to include baseline endoscopic ultrasonography, computerized tomography of the chest and abdomen, complete blood count, serum chemistries, pulmonary function studies, and electrocardiogram. All patients were evaluated and discussed by the multidisciplinary team (comprising of various specialties: gastroenterology, pathology, medical oncology, radiation oncology, thoracic surgery, and others). All patients were a priori deemed eligible for and later completed TMT. The surgical specimen of each patient was scored by previously published methods (Chirieac et al., 2005; Wu et al., 2007) and designated as: pathCR, some response, or exCRTR. We focused on the two extremes of response (pathCR and exCRTR).

2.2. Trimodality therapy

All patients received 50.4 Gy of radiation in 28 fractions. Concurrent chemotherapy included a fluoropyrimidine plus either a platinum compound or taxane. Approximately 6 weeks after the completion of chemoradiation, patients underwent a

preoperative work-up to include imaging studies, blood tests, and upper endoscopy with biopsies. Surgery ensued. The type of surgery to be performed (Ivor-Lewis, transhiatal, or other) was at the discretion of the primary surgeon.

2.3. Follow-up of patients

After surgery, patients were followed periodically for 5 years or until death. Survival data were collected from our Tumor Registry, medical records, or the Social Security Database.

2.4. Tissue collection and analysis

Untreated tumor biopsies for research purposes were collected under an Institutional Review Board approved ongoing banking protocol at our institution. Research studies were performed under another approved protocol. Histology was confirmed in the corresponding adjacent section prior to ALDH-1 staining. All tissue sections were 4-μm in thickness and numerically adjacent. Staining was performed using Abcam ab23375 antibody, rabbit polyclonal at 1:100 dilution. Positive controls were placed on all tissue sections and consisted of FFPE cell pellets of cell lines known to overexpress ALDH-1. Negative controls were used as well. Two members of the team, without prior knowledge of patient outcome, independently scored each tumor to establish the average labeling index. Procedure was in place for discordant cases to be jointly reviewed under a double-headed microscope. Minimum of 200 and maximum of 400 tumor cells were counted in highest scoring region(s). Results were then submitted for analysis to our biostatisticians.

2.5. Cells and reagents

The human esophageal adenocarcinoma cell lines FLO-1, BE3, SKGT-4, JHESO and OACP (provided by Drs. Raju and Hung, both at our institution) (Raju et al., 2006; Soldes et al., 1999). All cell lines are authorized and re-characterized in the core facility every 6 months. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 mg/mL streptomycin and 100 IU/mL of penicillin). 5-FU and docetaxel were purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies ALDH-1 and Shh were obtained from Abcam (Cambridge, MA), YAP1 was purchased from Santa Cruz Biotechnology; SOX9 was from were purchased from Chemicon (Billerica, MS), Bcl-2 was obtained from Cell SignalingTechnology (Beverly, MA).

2.6. Flow cytometric labeling and fluorescence-activated cell sorting

ALDH-1 activity was assessed by fluorescence-activated cell sorting in three cell lines (OAPC, JHESO and FLO-1) according to the ALDEFLUOR detection kit following the protocol and Diethylaminobenzaldehyde (DEAB) was used to inhibit ALDH-1 activity to show the specificity of the detection. ALDH-1 positive or negative cells were sorted from JHESO cells by fluorescence-activated cell sorting according to the ALDE-FLUOR detection kit. ALDEFLUOR/DEAB treated cells were used to define negative gates. FACS was performed with $>1 \times 10^6$ cells using the BD FACSCanto II (Becton Dickinson) or FACSAria (Becton Dickinson).

2.7. Tumor sphere formation assay

Tumor sphere culture was performed as previously described (Song et al., 2013). Briefly, Single cells or FACS-isolated ALDH-1+ or ALDH-1− cell populations (2500/well) were seeded in triplicate onto a 6-well ultra-low attachment plate (Corning) in serum-free DMEM/F-12 supplemented with 10 ng/ml epidermal growth factor, 5 μg/ml insulin, 0.5 μg/ml hydrocortisonum and bovine pituitary extract (Invitrogen). After 10–14 days of culture, the number of tumor spheres formed (diameter >100 μm) was counted under a microscope.

2.8. Cell proliferation assay

Cell proliferation assays were performed using the CellTiter 96 aqueous nonradioactive cell proliferation assay (MTS) according to the instructions of the manufacturer (Promega Co., Madison, WI). All assays were performed in triplicate and repeated at least three times.

2.9. Protein extraction and western blot analysis

Protein isolation and Western blot analyses were performed as previously described and immunoreactive bands were visualized by chemiluminescence detection (Song et al., 2009).

2.9.1. Establishment of 5FU-resistant EC cells

The 5-FU resistant SKGT-4 and Yes-6 cell lines were generated by continuously culturing the drug-sensitive parental cell lines (SKGT-4 and Yes-6) in medium containing increasing concentrations of 5-FU in a stepwise procedure over 6 months. Resistant cell lines were maintained in the presence of 5-FU. To avoid an influence of 5-FU, all resistant cell lines were cultured in 5-FU-free medium for over 3 weeks before subsequent analysis.

2.9.2. Statistical methods

Univariate logistic regression was fit for the binary outcome of pathCR (or exCRTR), where ALDH-1 LI was included as the only covariate. The fitted model was validated through a 3-fold cross validation, where 2/3 of the data set was used as the "training set" and remaining 1/3 served as the "validation set". This process was repeated 1000 times and the average concordance index was summarized. The concordance index (C-index) (Harrell, 2001) is a measure for validating the predictive ability of a survival model and ranges between 0 and 1, with higher values indicating better predictive/discriminative models. We also assessed the goodness-of-fit through fitting the model using 1000 bootstrap samples. All statistical analyses were performed in Splus.

3. Results

3.1. Patient characteristics

Patient characteristics are shown in Table 1. The majority of patients were men (89%) and had adenocarcinoma histology (96%). Most had baseline T3 or higher tumors (84%) and node positive (61%) disease by endoscopic ultrasound.

Table 1 − Patient characteristics.		
		Frequency
Age (years)	Median	62
	Range	27−80
Gender	M	149	89.22%
	F	18	10.78%
Ethnicity	White	152	91.02
	Hispanic	13	7.78
	African−American	2	1.20
Histology	Adeno	160	95.81%
	SCC	7	4.19%
Tumor grade	Well-diff	4	2.40%
	Mod diff	72	43.11%
	Poorly diff	91	54.49%
Baseline EUST Stage	Tx	8	4.79%
	T2	19	11.38%
	T3	137	82.04%
	T4	2	1.20%
	T4b	1	0.60%
Baseline EUS N stage	N0	60	35.93%
	N1	102	61.08%
	Nx	5	2.99%
Baseline M	M0	155	92.81%
	M1a	12	7.19%
Induction Chemo	Yes	67	40.12
	No	100	59.88
Type of surgery	Ivor Lewis Esophagectomy	113	67.66
	Transhiatal Esophagectomy	18	10.77
	Transthoracic Esophagectomy	9	5.39
	Three-field Esophagectomy	13	7.78
	Minimally Invasive Esophagectomy	14	8.38
Taxanes[a]		96
Platinum[a]		86
Taxane and platinum[a]		21

EUS denotes, endoscopic ultrasonography.
[a] All patients received a fluoropyrimidine (iv or oral).

3.2. Response to chemoradiation

40 (24%) of 167 patients had pathCR, 27 (16%) had exCRTR and the rest had some evidence of response in the surgical specimen.

3.3. Overall and progression-free survival

Of 167 patients, 87 have died. The median overall survival (OS) is 46 months (95% CI: 32.6−79.6). Of 167 patients, 95 have died or experienced relapse. The median progression-free survival time is 28 months (95% CI: 19.5-not estimable).

3.4. Prediction: ALDH-1 labeling indices and response to chemoradiation

Figure 1 shows an example of ALDH-1 staining in untreated tissues of a patient with pathCR and another with exCRTR.

For pathCR, the partial residual plot suggested a linear association between ALDH-1 and the logit of pathCR (there was only one outlier with ALDH-1 LI of 0.6). The fitted univariate logistic regression model suggested that higher ALDH-1 LI was associated with lower probability of pathCR (Odds ration [OR] = 0.432; $p=<0.001$). The C-index was 0.797 for the fitted model. The 3-fold cross validation resulted in an average C-index of 0.798. The C-index from 1000 bootstrap samples was also 0.798 (Figure 2).

For exCRTR, the partial residual plot suggested a linear association between ALDH-1 and the probability of exCRTR (there was only one exception with ALDH-1 LI of 0.3). The fitted univariate logistic regression model suggested that higher ALDH-1 is associated with higher logit of achieving exCRTR (OR = 3.782; $p=<0.001$). The C-index was 0.961 for the fitted model. The 3-fold cross validation resulted in an average C-index of 0.961 and it was 0.960 from 1000 bootstrap samples (Figure 3).

3.5. Prognosis: ALDH-1 labeling indices and prognosis of patients

For overall survival, the Martingale residual plot suggested a linear relationship between ALDH-1 LIs and the risk of death. The fitted univariate Cox proportional hazard model suggested that higher ALDH-1 LI was significantly associated with an increased risk of death (Hazard ratio [HR] = 3.4; $p = 0.03$).

For progression-free survival, the Martingale residual plot suggested a linear relationship between ALDH-1 Li and the risk of death or relapse. The fitted univariate Cox proportional hazards model suggested that higher ALDH-1 LI was significantly associated with increase risk of death or relapse (HR = 3.87; $p = 0.006$). ALDH-1 expression was associated with lymph node metastases.

3.6. ALDH-1 and resistance in cell lines with upregulation of resistance-conferring genes

The proportion of ALDH-1+ cells is high for OAPC cells, medium for JHESO cells and low for FLO-1 cells (Figure 4). FACS analysis demonstrated that the level of ALDH1+ cells in EC cell lines are correlated with response to 5-FU (data shown) and docetaxel (data not shown) treatment. The cells with higher proportion of ALDH-1+ such as OACP and JHESO were more resistant to chemotherapy than FLO-1 cells with lower proportion of ALDH-1+. Correspondingly, stem cell signaling related genes: SOX9, Shh and Hes-1 which have been reported as resistance conferring genes are correlated to ALDH1 level as well as response to 5-FU and docetaxel.

ALDH-1+ and − cells were sorted from JHESO cells. ALDH-1+ cells had a higher proliferation rate than ALDH-1− cells. ALDH-1− cells were highly sensitive to 5-FU compared to ALDH-1+ cells (Figure 5).

Our results also demonstrated that cell lines with higher ability to form tumor spheres (OAPC and JHESO) had a higher fraction of ALDH-1+ cells and were more resistant to 5-FU and docetaxel compared to those with diminished ability to form tumor spheres (SKGT-4, BE3, and FLO-1; Supplemental Figure 1).
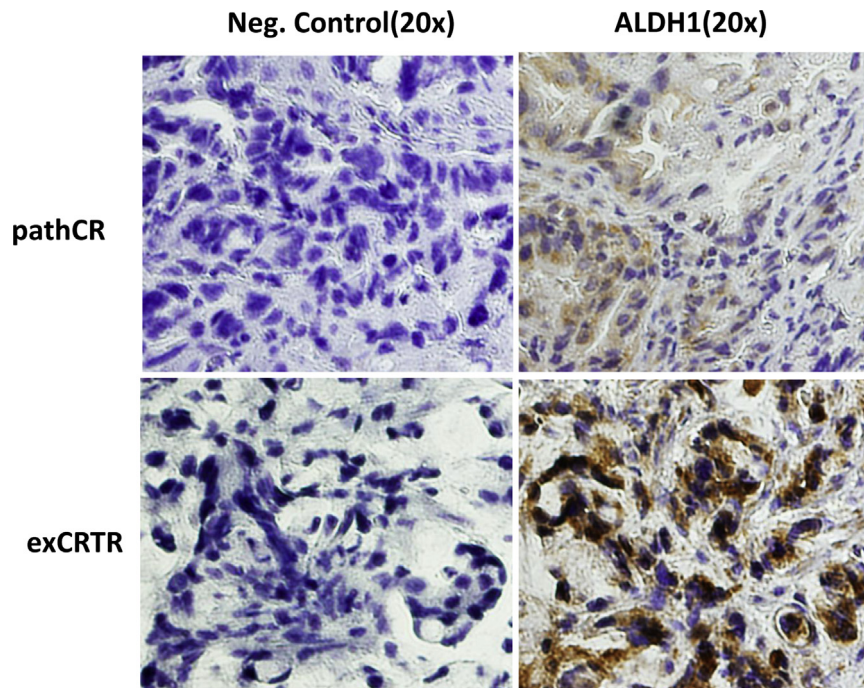
Figure 1 – Immunohistochemistry staining of ALDH-1 in untreated EC tumor of a patient with pathCR showing low LI (upper row) and a patient with exCRTR showing high LI (lower row). Abbreviations: pathCR, pathologic complete response; exCRTR, extreme resistance; and LI, labeling index.

Upregulation of *ALDH-1* and other stem cell markers (*YAP1* and *Gal-3*) were noted in SKGT-4 and YES-6 cells when both were made resistant to 5-FU (Supplemental Figure 2).

4.   Discussion

Our results on EC cells suggest that high ALDH-1 LIs are associated with therapy resistance, aggressive phenotype (higher proliferative rate) in tumor spheres, and overexpression of resistance conferring genes (*Shh*, *YAP1*, *Shh*, *Gal-3*, and *Hes-1*). In addition, we observed that if cells were made resistant to 5-FU, the proportion of ALDH-1+ cells increased, there was overexpression of resistance conferring genes, and aggressive phenotype in the tumor sphere assays. These unique data support the observations made in our large cohort of patients.
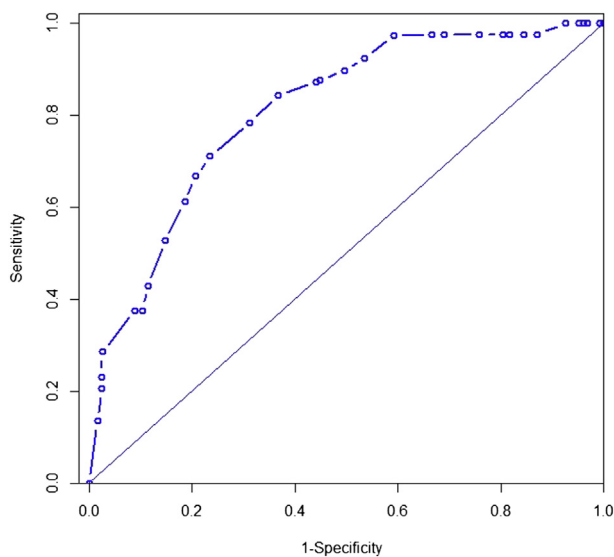

Figure 2 – Area under the receiver operating characteristic curve for pathCR (pathologic complete response) with C-index of 0.798.
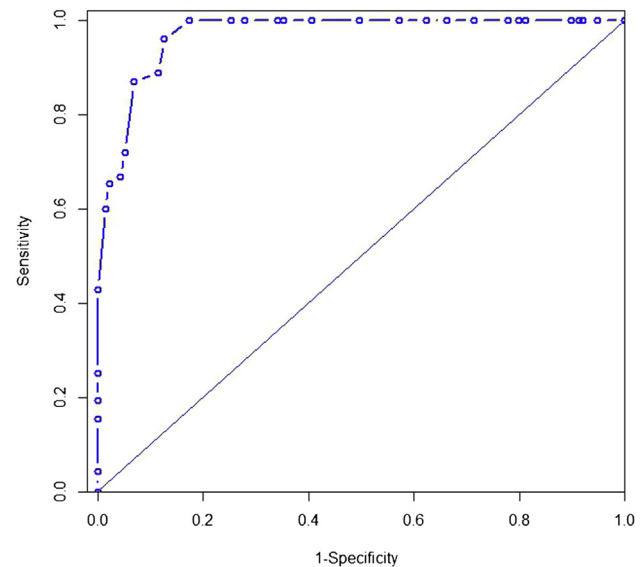

Figure 3 – Area under the receiver operating characteristic curve for exCRTR (pathologic extreme resistance) with C-index of 0.960.
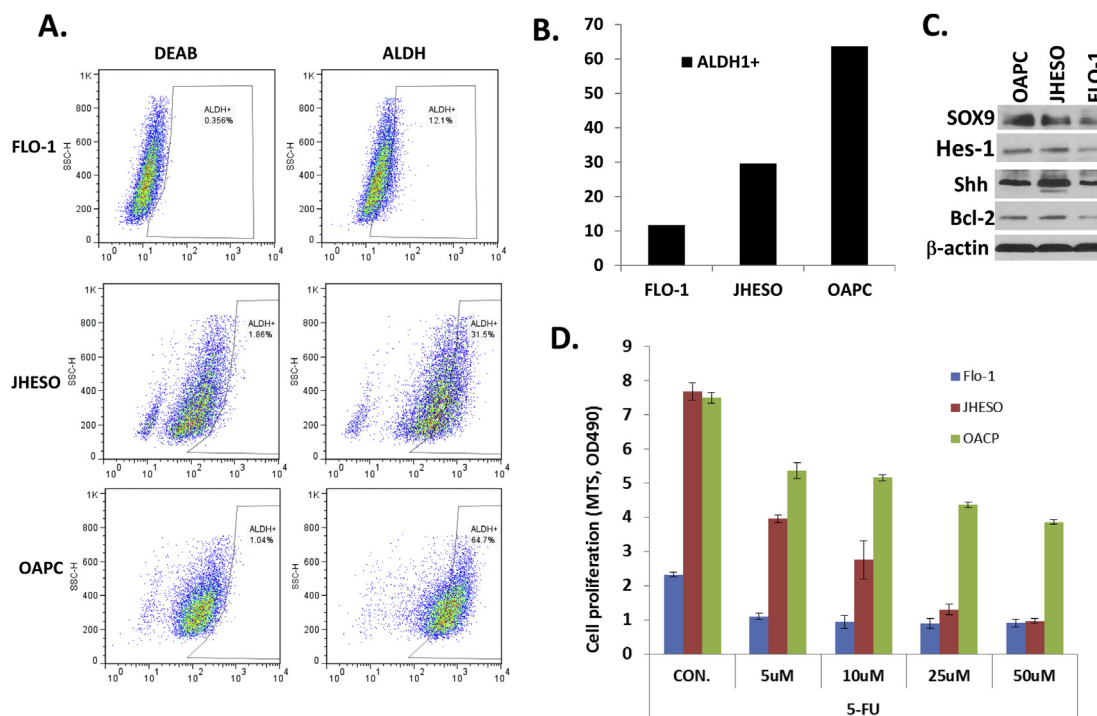
Figure 4 − EC cells with high ALDH-1 LIs are resistant to chemotherapy and upregulate resistance-conferring genes. A. ALDH1 activity assessed by fluorescence activated cell sorting in 3 cell lines (FLO-1, JHESO, and OAPC) according to ALDEFLUOR detection kit following the protocol and diethylaminobenzaldehyde (DEAB) was used to inhibit ALDH-1 activity to document the detection specificity. B. Bar graphs demonstrating the percentage of ALDH-1 positive cells in 3 cell lines. C. Immunoblotting of 3 cell lines demonstrates upregulation of resistance conferring CSC genes are more upregulated in cells with higher percentage of ALDH-1. D. 3 cell lines exposed to various concentrations of 5-FU and the degree of response (cells with high percentage of ALDH-1 are more resistant).

Our cohort of 167 was treated with the uniform strategy of TMT. The pathologic responses were characterized by validated method (Wu et al., 2007) and ALDH-1 LIs were assessed in untreated tumor tissue. Our data are particularly striking for the enrichment of only exCRTR tumor patients in a specific category where ALDH-1 LI are ≥60. The C-index in various models was 0.960 by two cross validation methods. This observation opens up a possibility of a therapeutic strategy in which one could avoid chemoradiation prior to surgery in these patients or add a specific agent that might overcome resistance to chemoradiation; e.g., Shh inhibition (Sims-Mourtada et al., 2007) or by targeting other genes such asYAP1, Gal-3, Hes-1, etc. The data on patients who achieved pathCR is also encouraging. Nearly all pathCR patients had ALDH-1 LI < 0.3 (there was only one exception). The C-index for pathCR was 0.798 by two cross validation methods. This observation could also trigger a unique strategy. Patients who have low baseline ALDH-1 LI (and achieve a clinical complete response as defined in the reference (Cheedella et al., 2012) following completion of chemoradiation) could be investigated for selective surgery.

Our results define the heterogeneity of response to chemoradiation in patients with localized EC and, tie these results to ALDH-1 (a well known marker of CSCs or side population). Our results are supported by complimentary findings that higher the density of CSCs in a given tumor, higher is the likelihood of the tumor being resistant to therapy (Steg et al., 2012). In addition, our non-clinical data demonstrate that upregulation of resistance conferring genes occurs when cells have high ALDH-1 LIs or are made resistant to cytotoxic agents (which also enriches for ALDH-1+ cells). These observations provide a research trajectory to focus on a specific therapeutic target(s) in patients that can be exploited to overcome therapy resistance. Most importantly, these are the first data to establish ALDH-1 as a predictive biomarker.

Our analysis has the drawback of not having an independent validation cohort yet. This would be our next step before strategizing for clinical implementation. We were able to cross-validate by two separate methods and provide unique non-clinical supportive data. The strength of our results is that (1) this is the first report of ALDH-1 as a predictive marker in cancer patients, (2) we are reporting on a large number of patients all treated with TMT, (3) we have scored the surgical specimens for treatment response assessment with a validated method, (Wu et al., 2007) and (4) our non-clinical data confirm the clinical observations but also provide insights into how we could uncover clinical therapeutic targets for EC patients.

5.   Conclusion

In conclusion, ALDH-1 LIs are predictive of pathCR ($p \leq 0.001$) and exCRTR ($p \leq 0.001$), and prognostic of overall survival
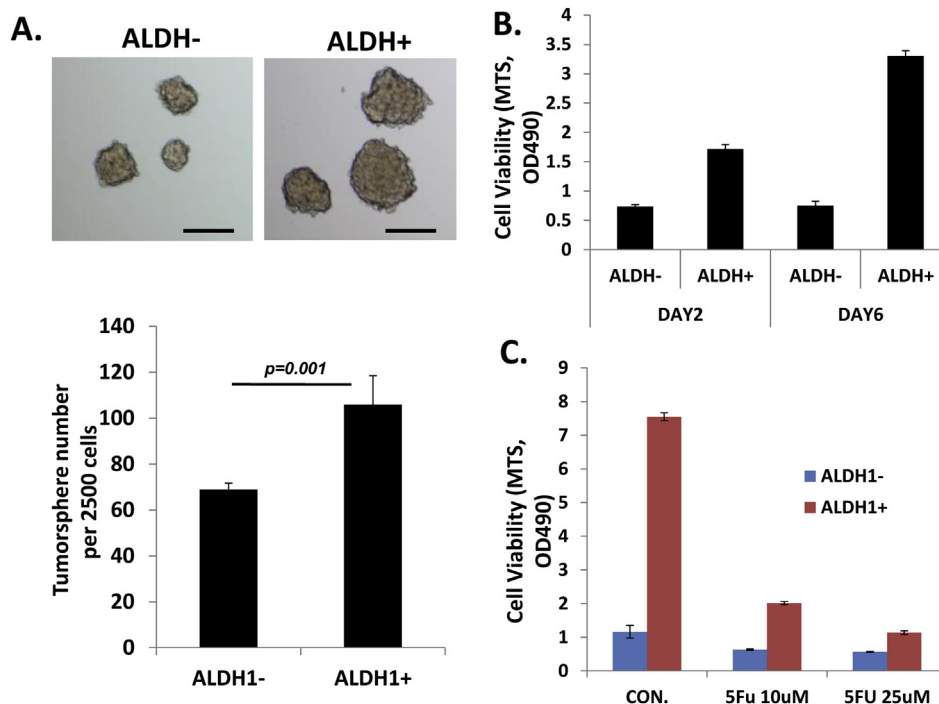
Figure 5 − Cells with higher percentage of ALDH-1 more readily for tumor sphere and are resistant to 5-FU. A. From JHESO, ALDH-1+ and ALDH-1− cells are sorted by fluorescence activated cell sorting in 3 cell lines (FLO-1, JHESO, and OAPC) according to ALDEFLUOR detection kit following the protocol and tumor sphere assays were carried out in triplicate in ultra-low attachment plate in the tumor sphere medium. After 8−10 days in culture, the number and size of the tumor spheres were counted under a microscope. The ALDH-1+ cells formed larger tumor spheres and these are numerically shown in the bar graph below. B. The proliferation rate of ALDH1+ and ALDH-1− cells using the MTS proliferation assay on day 2 and day 6 demonstrate higher proliferative activity of ALDH-1+ cells. C. ALDH-1+ cells are more resistant to 5-FU at different concentrations.

($p = 0.03$) and progression-free survival ($p = 0.006$), produce high C-index by two cross validation methods for exCRTR (0.960) and pathCR (0.798), and finally, our non-clinical data parallel our clinical observations and provide insights into how to navigate effectively for making more progress. Finally, our data can pave the path to implementation of a biomarker strategy for individualized therapeutic strategies for EC patients.

Conflict of interest

The authors have declared no conflict of interest.

Appendix A.
Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molonc.2013.10.007.

REFERENCES

Ajani, J.A., Barthel, J.S., Bentrem, D.J., et al., 2011. Esophageal and esophagogastric junction cancers. J. Natl. Compr. Canc Netw. 9, 830−887.

Ajani, J.A., Correa, A.M., Hofstetter, W.L., et al., 2012. Clinical parameters model for predicting pathologic complete response following preoperative chemoradiation in patients with esophageal cancer. Ann. Oncol. 23, 2638−2642.

Allum, W.H., Stenning, S.P., Bancewicz, J., Clark, P.I., Langley, R.E., 2009. Long-term results of a randomized trial of surgery with or without preoperative chemotherapy in esophageal cancer. J. Clin. Oncol. 27, 5062–5067.

Awad, O., Yustein, J.T., Shah, P., et al., 2010. High ALDH activity identifies chemotherapy-resistant Ewing's sarcoma stem cells that retain sensitivity to EWS-FLI1 inhibition. PLoS ONE 5, e13943.

Brennan, S.K., Meade, B., Wang, Q., Merchant, A.A., Kowalski, J., Matsui, W., 2010. Mantle cell lymphoma activation enhances bortezomib sensitivity. Blood 116, 4185–4191.

Cheedella, N.K., Suzuki, A., Xiao, L., et al., 2012. Association between clinical complete response and pathological complete response after preoperative chemoradiation in patients with gastroesophageal cancer: analysis in a large cohort. Ann. Oncol. 24, 1262–1266.

Chirieac, L.R., Swisher, S.G., Ajani, J.A., et al., 2005. Posttherapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer 103, 1347–1355.

Ginestier, C., Hur, M.H., Charafe-Jauffret, E., et al., 2007. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Null. 1, 555–567.

Harrell, F.E., 2001. Regression Modeling Strategies. Springer-Verlag, New York.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA Cancer J. Clin. 61, 69–90.

Jiang, F., Qiu, Q., Khanna, A., et al., 2009. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol. Cancer Res. 7, 330–338.

Kelsen, D.P., Ginsberg, R., Pajak, T.F., et al., 1998. Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. N. Engl. J. Med. 339, 1979–1984.

Kim, M.P., Fleming, J.B., Wang, H.M., et al., 2011. ALDH activity selectively defines an enhanced tumor-initiating cell population relative to CD133 expression in human pancreatic adenocarcinoma. PLoS ONE 6.

Moreb, J.S., Baker, H.V., Chang, L.J., et al., 2008. ALDH isozymes downregulation affects cell growth, cell motility and gene expression in lung cancer cells. Mol. Cancer 7, 87.

Nguyen, G.H., Murph, M.M., Chang, J.Y., 2011. Cancer stem cell Radioresistance and enrichment: where frontline radiation therapy may fail in lung and esophageal cancers. Cancers (Basel) 3, 1232–1252.

Nogami, T., Shien, T., Tanaka, T., et al., 2012. Expression of ALDH1 in axillary lymph node metastases is a prognostic factor of poor clinical outcome in breast cancer patients with 1-3 lymph node metastases. Breast Cancer. Epub ahead of print, PMID: 2247396.

Raju, U., Ariga, H., Koto, M., et al., 2006. Improvement of esophageal adenocarcinoma cell and xenograft responses to radiation by targeting cyclin-dependent kinases. Radiother. Oncol. 80, 185–191.

Ricardo, S., Vieira, A.F., Gerhard, R., et al., 2011. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J. Clin. Pathol. 64, 937–946.

Rice, T.W., Rusch, V.W., Apperson-Hansen, C., et al., 2009. Worldwide esophageal cancer collaboration. Dis. Esophagus 22, 1–8.

Rizk, N.P., Venkatraman, E., Bains, M.S., et al., 2007. American joint committee on cancer staging system does not accurately predict survival in patients receiving multimodality therapy for esophageal adenocarcinoma. J. Clin. Oncol. 25, 507–512.

Rohatgi, P., Swisher, S.G., Correa, A.M., et al., 2005a. Characterization of pathologic complete response after preoperative chemoradiotherapy in carcinoma of the esophagus and outcome after pathologic complete response. Cancer 104, 2365–2372.

Rohatgi, P.R., Swisher, S.G., Correa, A.M., et al., 2005b. Failure patterns correlate with the proportion of residual carcinoma after preoperative chemoradiotherapy for carcinoma of the esophagus. Cancer 104, 1349–1355.

Sims-Mourtada, J., Izzo, J.G., Apisarnthanarax, S., et al., 2006. Hedgehog: an attribute to tumor regrowth after chemoradiotherapy and a target to improve radiation response. Clin. Cancer Res. 12, 6565–6572.

Sims-Mourtada, J., Izzo, J.G., Ajani, J., Chao, K.S., 2007. Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport. Oncogene 26, 5674–5679.

Soldes, O.S., Kuick, R.D., Thompson 2nd, I.A., et al., 1999. Differential expression of Hsp27 in normal oesophagus, Barrett's metaplasia and oesophageal adenocarcinomas. Br. J. Cancer 79, 595–603.

Song, S., Mazurek, N., Liu, C., et al., 2009. Galectin-3 mediates nuclear beta-catenin accumulation and Wnt signaling in human colon cancer cells by regulation of glycogen synthase kinase-3beta activity. Cancer Res. 69, 1343–1349.

Song, S., Maru, D.M., Ajani, J.A., et al., 2013. Loss of TGF-beta adaptor beta2SP activates notch signaling and SOX9 expression in esophageal adenocarcinoma. Cancer Res. 73, 2159–2169.

Steg, A.D., Bevis, K.S., Katre, A.A., et al., 2012. Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. Clin. Cancer Res. 18, 869–881.

Thrift, A.P., Whiteman, D.C., 2012. The incidence of esophageal adenocarcinoma continues to rise: analysis of period and birth cohort effects on recent trends. Ann. Oncol. 23, 3155–3162.

van Hagen, P., Hulshof, M.C., van Lanschot, J.J., et al., 2012. Preoperative chemoradiotherapy for esophageal or junctional cancer. N. Engl. J. Med. 366, 2074–2084.

Wang, Y.C., Yo, Y.T., Lee, H.Y., et al., 2012a. ALDH1-Bright Epithelial ovarian cancer cells are associated with CD44 expression, drug resistance, and poor clinical outcome. Am. J. Pathol. 180, 1159–1169.

Wang, Y., Zhe, H., Gao, P., Zhang, N., Li, G., Qin, J., 2012b. Cancer stem cell marker ALDH1 expression is associated with lymph node metastasis and poor survival in esophageal squamous cell carcinoma: a study from high incidence area of northern China. Dis. Esophagus 25, 560–565.

Wu, T.T., Chirieac, L.R., Abraham, S.C., et al., 2007. Excellent interobserver agreement on grading the extent of residual carcinoma after preoperative chemoradiation in esophageal and esophagogastric junction carcinoma: a reliable predictor for patient outcome. Am. J. Surg. Pathol. 31, 58–64.

Zhang, G., Ma, L., Xie, Y.K., Miao, X.B., Jin, C., 2012. Esophageal cancer tumorspheres involve cancer stem-like populations with elevated aldehyde dehydrogenase enzymatic activity. Mol. Med. Rep. 6, 519–524.