Aldehyde dehydrogenase 1 isoenzyme expression as a marker of cancer stem cells correlates to histopathological features in head and neck cancer: A meta-analysis

Yue Dong1*, Sebastian Ochsenreither2*, Chengxuan Cai1, Andreas M. Kaufmann3, Andreas E. Albers4*, Xu Qian1*

1 Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical University, Wenzhou, P.R. China, 2 Hematology, Oncology and Tumorimmunology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany, 3 Clinic for Gynecology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany, 4 Department of Otorhinolaryngology, Head and Neck Surgery, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany

* These authors contributed equally to this work.
* andreas.albers@charite.de (AEA); Qian_michelle2014@163.com (XQ)

Abstract

There is a lack of predictive biomarkers that can identify patients with head and neck squamous cell carcinoma (HNSCC) who will experience treatment failure and develop drug resistance, recurrence, and metastases. Cancer stem-like cells (CSC) were identified as a subset of cells within the tumor in a variety of solid tumors including HNSCC. CSC are considered the tumor-initiating population responsible for recurrence or metastasis and are associated with therapy resistance. This meta-analysis including fourteen studies with altogether 1258 patients updates and summarizes all relevant data on the impact of ALDH1+ CSC on the prognosis of HNSCC and its association with clinicopathological parameters. ALDH1 expression is highly correlated with tumor differentiation (G3 vs. G1+G2; odds ratio = 2.85. 95% CI: 1.72–4.73, P<0.0001) and decreased overall survival (relative risk = 1.77. 95% CI: 1.41–2.22, P<0.0001) if one out of seven studies was excluded because of heterogeneity. These findings provide insights into the understanding of more aggressive tumor phenotypes and also suggest that the prognostic value provided by HNSCC-subtyping by CSC frequency warrant further clinical investigation.

Introduction

Recent discoveries in cancer research continue to support the theory that cancer stem cells (CSC) initiate metastasis formation and tumor recurrence after initial therapy [1, 2]. Such a cancer initiating cell population has not only been described for head and neck squamous
cell carcinoma (HNSCC) but also proved being relevant for clinical outcome of patients with such a disease as observed by our group and others [3–5]. HNSCC is regarded one of the most prevalent malignant diseases worldwide. Multimodal treatment strategies have substantially improved survival in patients with curative potential [6–9]. However, treatment decision in clinical routine especially for locally advanced (LA) HNSCC remains a challenge and is based on conventional factors such as tumor stage, comorbidities and a patient’s responses to ongoing treatments [10]. Among patients who had LA diseases and even with similar histopathological characteristics, diversity in responses to the same treatment regimen resulting also in differences in prognosis suggests so far unknown determining parameters [11]. Thus, additional stratification factors could augment the TNM staging system to improve prediction of long-term survival. That is, biomarker stratified treatment strategies could become a reality. Although HNSCC prognosis, and specifically in oropharyngeal cancer (OPSCC), has clearly been influenced by detection of human papilloma virus (HPV) infection [12], additional biomarkers are needed for HPV-negative tumors. Furthermore, in case CSC have a predictive or prognostic role for patients with HNSCC as suggested by preclinical studies, the targeting of the CSC cell population in combination with conventional therapies aiming at a reduction or eradication of the bulk tumor would be a reasonable approach to improve therapeutic efficacy by prevention of local recurrence or metastasis formation.

CSC can be defined by their biological features like unlimited proliferative capacity, and enhanced resistance to conventional treatment modalities like chemotherapy or radiation, by phenotypical markers like receptor expression, or by indirect detection of specific transmembrane transporter molecules. Among these CSC markers, the aldehyde dehydrogenase 1 (ALDH1) isoenzymes, which can be detected by their enzymatic function as well as by immunohistochemistry, are well studied in HNSCC. ALDH1 is associated with a stem cell-like phenotype, which is among other mechanisms a direct consequence of its obvious activity for oxidation of retinal to retinoic acid leading to cell differentiation [13]. Furthermore, ALDH1 expression enhances resistance to several anticancer drugs by its catalytic activity [14]. In cell lines, ALDH1-positivity is associated with tumorigenesis and resistance to chemo-radiation [4, 15]. ALDH1A1 positive CSCs are found both in primary tumors originating from the oral cavity, oropharynx, hypopharynx and larynx [5] and in lymph node metastases [16, 17]. Clinical studies have shown the prognostic value of ALDH1A1 in patients with LA HNSCC as well as in patients with nodal and visceral metastatic disease [5, 17].

Because of the persistence of CSC during therapy, which contributes to tumor relapse and its capacity for recapitulation of the heterogeneous features of a cancer, eradication of CSC is viewed as an important challenge for a successful cancer therapy. In a recent ex vivo study, NCT-501, a theophylline-based inhibitor of ALDH1A1, was able to overcome secondary resistance to several chemotherapeutic agents. The combination with cisplatin treatment was found to significantly decrease the proliferation of primary HNSCC cells compared to treatment with the individual agents [18]. Moreover, ALDH1A1 has been proven to be immunogenic and a target for cytotoxic T-cells. It has been shown that T-cell lines specific for MHC class I-restricted epitopes of ALDH1A1 can eliminate ALDH1A1 positive cancer cells in HNSCC [19, 20]. These observations imply a clinical usefulness of ALDH1A1 not only as a predictive biomarker but as potential target in HNSCC.

In order to assess clinical and biological characteristics such as histological appearance, lymph node metastasis and patient’s survival in relation to ALDH1 expression in HNSCC, we conducted a systematic review of the literature and meta-analysis. Strategies targeting ALDH1 positive CSC are also discussed.
Materials and methods

Literature search strategy and selection criteria

Electronic literature databases PubMed, EMBASE, the Cochrane Library, and WangFang databases for studies published before December 31st, 2016 were searched using the following key words: “ALDH1” or “aldehyde dehydrogenase 1” and “head and neck squamous cell” or “oral” or “laryngeal” or “pharyngeal” or “tongue” or “oropharyngeal” and “cancer” or “carcinoma” or “neoplasms”. Titles and abstracts were scanned to identify the candidate publications. Systematic reviews, letters, and case studies were excluded. Manuscripts from the reference lists of original or review articles were also screened for additional publications. In a second step, full-text articles were assessed to check for eligibility. To be included in the current study, original articles were required to meet the following criteria: (1) patients with HNSCC; (2) correlations between ALDH1 overexpression and histopathological parameters, overall survival (OS) or disease-free survival (DFS) of HNSCC patients were analyzed.

Data extraction

Pivotal data were extracted from all eligible publications independently by two of the authors (DY and QX). For this, data tables were composed to extract all relevant data from texts, tables and figures including patient characteristics, tumor stages and additional clinical features together with clinical endpoints like OS and DFS. Additionally, for each article, the authors, the year of publication, and the number of patients were extracted. To digitalize and extract survival data from Kaplan–Meier plot curves, the software GraphClick (Version 3.0.2, Arizona Software 2010, http://www.arizona-software.ch/graphclick) was used.

Study quality

The Newcastle-Ottawa Scale (NOS), a risk of bias assessment tool for observational studies, was used to evaluate the methodological quality of included studies [21, 22]. The quality assessment values ranged from 0 to 9 points. There are three categories including selection (4 points), comparability (2 points), and exposure (3 points) (S2 Table). The result was an overall risk of bias rating of each study and score ≥5 is considered high quality [22]. Two independent reviewers evaluated the risk of bias for each study.

Statistical analysis

All statistical analyses were performed using the methods described in the Cochrane Handbook for Systematic Review of Interventions [23], using the Reviewer Manager Software, version 5.3 (Cochrane Library, Oxford, UK) and STATA 12.1 (StataCorp LP, Texas, USA). The impact of ALDH1 overexpression on dichotomous data was expressed as the odds ratio (OR) and risk ratio (RR) with 95% Confidence Intervals (CI). OR was used to assess the association between ALDH1 overexpression and clinicopathological parameters such as clinical stage including lymph node status and tumor histology. RR was used to assess the association between ALDH1 overexpression and outcome (OS and DFS) [24].

We assessed the statistical heterogeneity by observing I-squared ($I^2$) and Q test [25, 26]. If a significant heterogeneity existed ($I^2 >50\%$ or $P <0.05$), a random effect model was used to calculate OR and RR. Otherwise a fixed effect model was used [26]. In order to explore the possible sources of heterogeneity, subgroup analyses and sensitivity analyses were conducted. In addition, Egger’s test was used to evaluate the publication bias [27].
Results

Description of the included studies

155 studies were retrieved from the databases and 14 studies [5, 16, 17, 28–38] met all predefined inclusion criteria and therefore were included in the meta-analysis (Fig 1). The representative search strategies are illustrated in S1 File. The total number of patients included was 1258. The main characteristics of the eligible studies are summarized in Table 1. Ten articles provided clinicopathological features, seven studies included data on OS and four studies included data on DFS. The median follow-up of 7 studies is illustrated in Table 1. The patient population of most studies was rather heterogeneous with patients of all clinical stages. Twelve studies did not include patients with distant metastases. The percentage of patients with distant metastases in the residual studies was 50% to 87.9% [16, 30]. HPV status was detected in four studies. Subsites of primary tumors of twelve studies are illustrated in S1 Table including 413 oral cancers, 509 OPSCC and 177 at other sites. Information on treatment modalities was provided in only a single study. Therefore, treatment was not included in our statistical analysis.

Correlation of ALDH1 expression with clinicopathological parameters

All studies used immunohistochemistry (IHC) with cut-off values between any staining to >10% positivity. Isoenzymes of ALDH1 are e.g., ALDH1A1, ALDH1A2, and ALDH1A3. The antibodies used, when described, were specific for all isoforms of ALDH1 in four studies, and ALDH1A1 in ten studies. Therefore, we used the term “ALDH1” in our analysis. The association of ALDH1 expression with clinicopathological parameters is illustrated in Fig 2. ALDH1 expression was associated with higher differentiation grade (G3 vs. G1+G2; OR = 2.85, 95% CI: 1.72–4.73, P<0.0001, fixed effect; Fig 2A) but not clinical stage (III+IV vs. I+II; OR = 1.34, 95% CI: 0.71–2.55, P = 0.37, fixed effect; Fig 2B). Sensitivity analysis showed stable results for differentiation (S1 Fig). Furthermore, expression of ALDH1 was not significantly associated...
with positive lymph node status (Pos vs. Neg; OR = 1.93, 95% CI: 0.98–3.79, P = 0.06, random effect; Fig 2C) or T-stage (T3+T4 vs. T1+T2; OR = 0.99, 95% CI: 0.71–1.38, P = 0.96, fixed effect; Fig 2D). ALDH1 expression was analyzed in four studies regarding the tumor sites. The studies of Koukourakis et al. and Ota et al. showed no significant relation of ALDH antibody 

ALDH1 expression and disease-free survival

DFS was investigated in 4 studies including 368 patients [17, 31–33]. We performed a meta-analysis on the DFS of ALDH+ and ALDH- patients. There was no significant association between ALDH1 expression and DFS (RR = 1.05; 95% CI: 0.35–3.16; P = 0.94, random effect; Fig 3A), again with significant heterogeneity (P<0.0001; I² = 88%). Then, we performed a sensitivity analysis where omission of each single article’s data did not result in obvious change in heterogeneity.

ALDH1 expression and 5-year overall survival outcome

We analyzed the OS of 672 patients from seven articles [5, 17, 29, 30, 33, 36, 37]. Using the DerSimonian–Laird fixed effects model, the meta-analysis on the OS of ALDH+ and ALDH- patients did not reach a statistical significance (RR = 1.44; 95% CI: 0.93–2.23, P = 0.10, random effect; Fig 3B). The forest plot showed a high level of heterogeneity, which was significant (I-

Table 1. Main characteristics and results of the eligible studies.

| Author          | Year | Country | Number of Patients | Site  | Tumor Stage (UICC) | Antibody                          | Cut-off score (H/L) | HPV status | Median follow-up |
|-----------------|------|---------|--------------------|-------|--------------------|-----------------------------------|---------------------|------------|-----------------|
| Chen YW [29]    | 2010 | Taiwan  | 111                | HNSCC | ND                 | ND, Abcam                         | ND                  | ND         | 75 months       |
| Koukourakis MI  | 2012 | Greece  | 74                 | HNSCC | I-III              | Rabbit mAb, EP1933Y, Abcam       | 5%                  | ND         | 24 months (4–80 months) |
| Michifuri Y [34]| 2012 | Japan   | 80                 | OSCC  | I-III              | Mouse mAb, clone 44, BD Pharmingen | 2%                  | ND         |                 |
| Xu J [17]       | 2012 | USA     | 96                 | HNSCC | I-IV               | N-19, Santa Cruz                  | 0                   | ND         | 62 months (12–150 months) |
| Liu W [32]      | 2013 | China   | 141                | OSCC  | ND                 | Rabbit mAb, ab52492, Abcam       | 5%                  | ND         | 66 months       |
| Chen C [39]     | 2013 | China   | 60                 | HNSCC | ND                 | ND, BD Biosciences                | ND                  | ND         |                 |
| Qian X [16]     | 2013 | Germany | 80                 | OPSCC | I-IV               | Mouse mAb, clone 44, BD Biosciences | 5%                  | Yes        |                 |
| Ota N [35]      | 2014 | Japan   | 90                 | OSCC  | I-IV               | Mouse mAb, clone 44; BD Biosciences | 5%                  | ND         |                 |
| Zhang M [36]    | 2014 | USA     | 222                | OPSCC | ND                 | Mouse mAb, clone 44; BD Biosciences | ND                  | Yes        |                 |
| Huang CF [30]   | 2014 | China   | 66                 | TSCC  | I-IV               | Polyclonal rabbit Ab, Proteintech Group Inc. | 10%             | ND         | 52 months (2–104 months) |
| Qian X [5]      | 2014 | Germany | 81                 | HNSCC | I-III              | Mouse mAb, clone 44; BD Biosciences | 5%                  | Yes        |                 |
| Leinung M [37]  | 2015 | Germany | 48                 | HNSCC | ND                 | Rabbit mAb, ab52492; Abcam       | ND                  | Yes        | 120 months      |
| Martin M [33]   | 2016 | Spain   | 57                 | LSCC  | I-IV               | ND, Abcam                         | ND                  | ND         | 42 months       |
| de Moraes FP    | 2016 | Brazil  | 52                 | HNSCC | I-IV               | EP1933Y, Abcam                    | 10%                 | ND         |                 |

IHC: immunohistochemistry; HNSCC: head and neck squamous cell carcinoma; OSCC: oral squamous cell carcinomas; TSCC: tongue squamous cell carcinoma; LSCC: laryngeal squamous cell carcinoma; OSCC: oral squamous cell cancer; OPSCC: oropharyngeal squamous cell carcinomas; ND: not documented.

https://doi.org/10.1371/journal.pone.0187615.t001
squared and Q-test, $P = 0.0002; I^2 = 77\%$). In order to show the individual influence of each study on the over-all heterogeneity, we performed a sensitivity analysis by which each study was removed from the pool of studies once and $I^2$ and $Q$ value was calculated. We demonstrated that the study of Zhang et al. [36] was the source of heterogeneity (S2 Fig). The removal of the latter study from the pooled population resulted in a significantly reduced $I^2$ value. Analysis of the modified patient population showed a conspicuous shorter OS in patients with positive ALDH1 expression (RR = 1.77, 95% CI: 1.41–2.22, $P < 0.0001$, random effect), which was illustrated in the modified forest plot (Fig 3C).

### Risk of bias of included studies and publication bias

Regarding to the NOS scale, all included studies had a score greater than 5 indicating a high quality of each study (S2 Table). Begg’s funnel and Egger’s test were used to access the publication bias for each item (Table 2). Egger’s test showed no significant publication bias for T-stage ($t = 0.70, P = 0.508$), lymph node status ($t = 1.01, P = 0.352$), differentiation grade ($t = 1.17, P = 0.306$), OS ($t = 0.83, P = 0.442$) and DFS ($t = -0.30, P = 0.792$).

### Discussion

ALDH1-expression is a commonly used marker identifying putative CSC in a variety of epithelial tumors directly or indirectly, to identify cells displaying resistance to conventional...
In this meta-analysis, the presence of ALDH1-positive cells in HNSCC was analyzed regarding tumor grading, stage at first diagnosis, and clinical outcomes. We showed that the presence of ALDH1-positive cells within primary tumors was strongly associated with a higher grading, which means with more dedifferentiated tumors.

It is hypothesized that putative stem cells are not a defined subpopulation within the malignant cells. The development from CSC to more differentiated cancer cells at the same tumor site or after dissemination is believed to be continuous. Specific embryonic stem cell-associated transcriptional regulators and gene expression pathways appear to be more upregulated in poorly differentiated but not in well differentiated tumors [40]. On a cellular level, some of these transcriptional factors associated with embryonic stem cells, e.g., Sox2, Oct3/4, and

### Table 2. Egger’s test of funnel plot asymmetry.

| Clinicopathological parameters       | t value | df  | p value |
|-------------------------------------|---------|-----|---------|
| Tumor grade                         | 1.17    | 5   | 0.306   |
| Lymph node metastasis               | 1.01    | 7   | 0.352   |
| Tumor T stage                       | 0.70    | 7   | 0.508   |
| Overall survival                    | 0.83    | 6   | 0.442   |
| Disease free survival               | -0.30   | 3   | 0.792   |

df: degrees of freedom.

https://doi.org/10.1371/journal.pone.0187615.t002
Nanog were significantly upregulated in ALDH1-positive CSC [41]. Accordingly, low-grade tumors should contain mostly differentiated cells and few CSC, and high-grade tumors should contain highly dedifferentiated cells exhibiting stem cell-like features and a high percentage of CSC, a notion which we proved to be true in our meta-analysis. Recently, the dedifferentiation from non-CSC into ZsGreen-cODC-positive CSC, a subpopulation of ALDH1-positive cells, was found in both HPV-negative and HPV-positive HNSCC cell lines in response to radiation [42]. Moreover, HPV-positive HNSCC cells that survived radiation dedifferentiated in CSC at lower rates while HPV-negative HNSCC had a higher rate. This phenomenon not only demonstrates that dedifferentiation exists during radiotherapy and thus the capacity of CSC for repopulation, but also explains in part the better survival for patients with HPV-positive HNSCC. However, very little is known of whether a dedifferentiation from non-CSC to CSC on a cellular level is possible. What is known is that epithelial-mesenchymal transition (EMT) and its reversed process mesenchymal-epithelial transition is related to the acquisition of stem cell properties. It appears that EMT can reprogram differentiated mammary epithelial cells into less differentiated epithelial stem cells with mesenchymal traits [43]. It also has been demonstrated in HNSCC that overexpression of ALDH1 dramatically decreased expression of epithelial markers but increased expression of mesenchymal markers [4] leading to enhanced invasiveness and metastatic potential [44]. A better understanding of how and why CSC are induced and modulated locally-regionally might suggest ways to target them.

HNSCC is used to describe all carcinomas arising from the stratified squamous epithelium lining the sinonasal tract, oral cavity, oropharynx, pharynx, and larynx. Histologically, squamous cell carcinoma is characterized by microscopic evidence of squamous differentiation and invasive growth. In most cases, the histological grading of these tumors is directly linked to the clinical stage at first diagnosis. At least for oral HNSCC, poorly differentiated tumors are more likely correlated with a positive nodal status, extracapsular spread, and perineural invasion [45]. Given the fact, that we found a clear association of ALDH1-expression with high histological grade, we expected an association of ALDH1 expression with clinical stage, T- and N-status. In a previous meta-analysis, Zhou et al. showed that the ALDH1 expression was significantly associated with lymph node metastasis, but not T-stage and the presence of distant metastases [46]. However, our analysis did not show significant impact of ALDH1 expression on disease stage. The discrepancy of the results may be due to the recent publications [33, 35, 36] enrolled in the dataset which have shown no significant correlations between lymph node metastasis and CSC. Although a significant correlation of ALDH1 CSC with nodal status was not concluded in this meta-analysis, in patterns of primary tumor and its corresponding nodal metastases, we (Qian et al.) showed that the proportion of ALDH1-expressing cells was significantly increased in nodal metastases compared to their corresponding primary tumors [16]. We also observed in vitro as a property of CSC an increased invasive capacity and expression of EMT-markers and decreased expression of adhesion molecule E-cadherin [4]. In addition, the EMT process can be reversed by transfer of miR34a-mimics into HNSCC cells [41]. We are currently planning a morphoproteomics-based prospective study to further investigate the outcome of HNSCC regarding CSC and EMT markers in order to tailor the possibility of biomarker-directed therapy.

In addition, HPV status may also be responsible for discrepant results. There was no correlation between ALDH1 expression and nodal metastases when only HPV+ tumors were included in the study of Leinung et al. [37]. The study by Zhang et al. [36] showed that HPV16+ OPSCC had a higher intrinsic CSC pool than HPV- OPSCC and no correlation was found between nodal status and ALDH1 positive CSC for the whole cohort. Because the authors did not perform the subgroup analysis based on HPV status, the frequency of CSC in HPV+ and HPV- tumors was not documented. According to the recent report of global
incidence of HPV infection in head and neck cancer [47], around 30% of OPSCC are caused by HPV, followed by oral cavity and larynx. The majority of subsites included in this meta-analysis are OPSCC, oral cavity and larynx. Thus, discrimination on HPV status to assess the effect of ALDH1 expression on lymph node metastasis is helpful to confirm or rebut this hypothesis.

The weakness of our meta-analysis is mainly the heterogeneity of studies and lack of clinical data. We could not find a statistical difference in DFS, which is a discrepancy compared to a previously published evaluation [46]. Indeed, several factors may account for the discrepancy of survival between two datasets. While the study by Liu et al. showed a clear signal for ALDH1 being associated with a shorter PFS, Martin et al. found the opposite result. Here it is important to note that the study by Liu et al. only included carcinomas of oral cavity [32]. Martin et al., on the other hand, included only laryngeal carcinomas with a portion of 26% stage IV patients [33]. Anatomic site, stage and histological characteristics are considered to be of importance for risk stratification of HNSCC [48–50]. As shown in a recent report by The Cancer Genome Atlas Network [51], increased expression of oxidative stress response genes associated with chemoresistance was found in laryngeal carcinomas while oral cavity tumors exhibit lower expression of genes related to DNA repair. Other confounding factors may include different ALDH1 testing methods used in each study and different treatment protocols. The prognostic impact of ALDH1 positivity on OS in HNSCC patients has been detected in seven of fourteen studies. When one source study was excluded, there was a significant impact of ALDH1 positivity on OS. Again, the observed heterogeneity of the patient populations is more likely to be responsible for the equivocal findings. The study by Zhang et al. [36] included exclusively HPV+ LA tumors which was associated with a favorable outcome than HPV− LA tumors [52]. Also, differences do exist in the CSC pool of HPV+ tumors and HPV− tumors [16, 42]. There are disagreements between the study of Zhang et al. where HPV+ HNSCC cells had a greater intrinsic CSC pool and other studies which showed a significantly lower frequency of CSCs in HPV+ tumors [42, 53]. In addition, because the patient samples enrolled in the Zhang study were microarrays, there would be limitations to conclude the results based on the selected tumor cores. They observed ALDH1 staining in >50% of the tumor cells in selected HPV− and HPV16+ OPSCC tumor cores. However, in our own study, the ALDH1 staining ranged from 0 to >50% in primary tumor and its corresponding nodal metastases. We also found higher ALDH1 expression grades and negative HPV status for primary tumors but not for metastases. These findings are consistent with studies in HNSCC cell lines which have shown HPV+ cell lines had lower numbers of CSCs [41] and inversely correlated with radiosensitivity [42]. Although these studies seem contradictory, it is worth noting that a small proportion of less radioresistant CSCs being identified in HPV-induced HNSCC were positively associated with decreased local regional control [54] indicating a contribution of CSC to the decreased local regional control in HPV+ tumors. The reason behind the observed differences warrants further investigations.

It is clear that CSC play crucial roles in tumorigenesis based on earlier observations, but currently its clinical relevance such as its proportions in pre- and post-treatment stage, response to treatment modalities and outcomes is still limited. The data revealed that ALDH1 expression is associated with tumor histology independent of HPV status indicating ALDH1-positive CSC as a potentially valuable tool in clinical management of HNSCC. The data also revealed the potentially prognostic value of ALDH1-positive CSC in HNSCC. It is necessary to further investigate the value of CSC presence as markers in stratified HNSCC subgroups based on factors such as HPV-association or tumor site. Treatment modalities targeting CSC populations in order to improve therapy are also currently of interest. Kulsum et al. demonstrated that ALDH1A1 inhibition improved the effectiveness of cisplatin treatment, reduced the migration rate, self-renewal capacity and tumorigenicity of cancer cells in vitro. Further, an ex
*vivo* study continuously showed that ALDH1A1-specific inhibitor in combination with cisplatin significantly decreased the proliferation of cells as compared to individual treatment [18]. Thus, strategies to introduce ALDH1A1-specific inhibitors to current chemoradiotherapy regimens, in an effort to target CSC and treatment-induced reprogramming, may become an ideal treatment to achieve better curative effects.

In conclusion, we have presented that ALDH1 expression is associated with tumor histology and has potential prognostic value independent of etiologies such as chronic alcohol, tobacco abuse, and HPV status in the meta-analysis. Additional limitations of our meta-analysis are the number of included articles and that the sample size of each study was rather small. There was heterogeneity of data among outcomes. We acknowledge that because of the limited data for our question of interest such as HPV status and tumor subsites were not discriminated, the strength of our conclusion is limited. Given the massive biases built in to case-control studies, ALDH1 as a putative biomarker should (at most) be presented as speculative. Future large-scale prospective studies to establish and validate the prognostic value of ALDH1 are necessary.

**Supporting information**

S1 Fig. Sensitivity analysis of ALDH1 expression with differentiation of HNSCC tissues. (TIF)

S2 Fig. Sensitivity analysis of ALDH1 expression with overall survival of HNSCC. (TIF)

S1 Table. Distribution of primary tumor sites of the eligible studies. (DOC)

S2 Table. Newcastle-Ottawa quality assessment scale of included studies. (DOCX)

S1 File. Search terms and the number of studies identified from Pubmed. (DOCX)

S2 File. PRISMA checklist. (DOC)

**Author Contributions**

**Formal analysis:** Yue Dong, Chengxuan Cai, Xu Qian.

**Funding acquisition:** Xu Qian.

**Methodology:** Yue Dong, Chengxuan Cai, Xu Qian.

**Resources:** Yue Dong.

**Supervision:** Sebastian Ochsenreither, Andreas M. Kaufmann, Andreas E. Albers, Xu Qian.

**Writing – original draft:** Yue Dong, Sebastian Ochsenreither, Andreas E. Albers, Xu Qian.

**Writing – review & editing:** Andreas M. Kaufmann, Andreas E. Albers, Xu Qian.

**References**

1. Qian X, Ma C, Nie X, Lu J, Lenarz M, Kaufmann AM, et al. Biology and immunology of cancer stem (-like) cells in head and neck cancer. Critical reviews in oncology/hematology. 2015; 95(3):337–45. Epub 2015/04/25. https://doi.org/10.1016/j.critrevonc.2015.03.009 PMID: 25907739.
2. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: ACR Workshop on cancer stem cells. Cancer research. 2006; 66(19):9339–44. Epub 2006/09/23. https://doi.org/10.1158/0008-5472.CAN-06-3126 PMID: 16990346.

3. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(3):973–8. Epub 2007/01/11. https://doi.org/10.1073/pnas.0610177104 PMID: 17210912.

4. Chen C, Wei Y, Hummel M, Hoffmann TK, Gross M, Kaufmann AM, et al. Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma. PloS one. 2011; 6(1):e16466. Epub 2011/02/10. https://doi.org/10.1371/journal.pone.0016466 PMID: 21304586.

5. Qian X, Wagner S, Ma C, Coorides A, Gekeler J, Klussmann JP, et al. Prognostic significance of ALDH1A1-positive cancer stem cells in patients with locally advanced, metastasized head and neck squamous cell carcinoma. Journal of cancer research and clinical oncology. 2014; 140(7):1151–8. Epub 2014/04/29. https://doi.org/10.1007/s00432-014-1685-4 PMID: 24770634.

6. Castaldi P, Rufini V, Bussu F, Micciche F, Dinapoli N, Autorino R, et al. Can “early” and “late” 18F-FDG PET-CT be used as prognostic factors for the clinical outcome of patients with locally advanced head and neck cancer? SpringerPlus. 2015; 4:208. Epub 2015/05/16. https://doi.org/10.1186/s40064-015-0988-5 PMID: 25977896.

7. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Cancer research. 2011; 71(1):e16466. Epub 2011/02/10. https://doi.org/10.1158/0008-5472.CAN-10-2482 PMID: 21428815.

8. Yoshida A, Hsu LC, Dave V. Retinal oxidation activity and biological role of human cytosolic aldehyde dehydrogenase 1A1-positive cancer stem cells by knockdown of Bmi-1. Oral oncology. 2010; 46(3):158–65. Epub 2009/12/29. https://doi.org/10.1016/j.oraloncology.2009.11.007 PMID: 20036608.

9. Chen YC, Chang CJ, Hsu HS, Chen YW, Tai LK, Tseng LM, et al. Inhibition of tumorigenicity and enhancement of radiochemosensitivity in head and neck squamous cell cancer-derived ALDH1-positive cells by knockdown of Bmi-1. Oral oncology. 2010; 46(3):158–65. Epub 2009/12/29. https://doi.org/10.1016/j.oraloncology.2009.11.007 PMID: 20036608.

10. Qian X, Wagner S, Ma C, Klussmann JP, Hummel M, Kaufmann AM, et al. ALDH1-positive cancer stem-like cells are enriched in nodal metastases of oropharyngeal squamous cell carcinoma independent of HPV status. Oncology reports. 2013; 29(5):1777–84. Epub 2013/03/14. https://doi.org/10.3892/or.2013.2340 PMID: 23483187.

11. Xu J, Muller S, Nannapaneni S, Pan L, Wang Y, Peng X, et al. Comparison of quantum dot technology with conventional immunohistochemistry in examining aldehyde dehydrogenase 1A1 as a potential biomarker for lymph node metastasis of head and neck cancer. Eur J Cancer. 2012; 48(11):1682–91. Epub 2012/02/22. https://doi.org/10.1016/j.ejca.2011.12.020 PMID: 22341992.

12. Kulsum S, Sudheendra HV, Pandian R, Ravindra DR, Siddappa G, R N, et al. Cancer stem cell mediated acquired chemoresistance in head and neck cancer can be abrogated by aldehyde dehydrogenase.
dehydrogenase 1A1 inhibition. Molecular carcinogenesis. 2017; 56(2):694–711. Epub 2016/07/07. https://doi.org/10.1002/mc.22526 PMID: 27380877.

19. Visus C, Ito D, Amoscato A, Maciejewska-Franczak M, Abdelsalem A, Dhir R, et al. Identification of human aldehyde dehydrogenase 1 family member A1 as a novel CD8+ T-cell-defined tumor antigen in squamous cell carcinoma of the head and neck. Cancer research. 2007; 67(21):10538–45. Epub 2007/11/03. https://doi.org/10.1158/0008-5472.CAN-07-1346 PMID: 17974998.

20. Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, Szczepanski MJ, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. Clinical cancer research: an official journal of the American Association for Cancer Research. 2011; 17(19):6174–84. Epub 2011/08/23. https://doi.org/10.1158/1078-0432.ccr-11-1111 PMID: 21856769.

21. Wells GA SB, O’Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohcr.ca/programs/c clinical_epidemiology/oxfordasp. 2014.

22. Peng F, Li H, Ning Z, Yang Z, Li H, Wang Y, et al. CD147 and Prostate Cancer: A Systematic Review and Meta-Analysis. PloS one. 2016; 11(9):e0163678. https://doi.org/10.1371/journal.pone.0163678 PMID: 27684938.

23. Higgins JP, Green S. Cochrane Handbook version 5.1.0. The Cochrane Collaboration. 2011.

24. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Statistics in medicine. 1998; 17(24):2815–34. Epub 1999/01/28. PMID: 9921604.

25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analyses detected by a simple, graphical test. BMJ (Clinical research ed). 1997; 315(7109):629–34. Epub 1997/10/06. PMID: 9310563.

26. Martin M, Hinojar A, Cerezo L, Garcia J, Lopez M, Prada J, et al. Aldehyde dehydrogenase isoform 1 (ALDH1) expression as a predictor of radiosensitivity in laryngeal cancer. Clin Transl Oncol. 2016; 18(8):825–30. https://doi.org/10.1007/s12094-015-1445-1 PMID: 26572760.

27. Michituri Y, Hirohashi Y, Torigoe T, Miyazaki A, Kobayashi J, Sasaki T, et al. High expression of ALDH1 and SOX2 diffuse staining pattern of oral squamous cell carcinomas correlates to lymph node metastasis. Pathol Int. 2012; 62(10):684–9. https://doi.org/10.1111/j.1440-1827.2012.02851.x PMID: 23005595.

28. Liu W, Wu L, Shen YM, Shi L, Zhang CP, Xu LQ, et al. Expression patterns of cancer stem cell markers ALDH1 and CD133 correlate with a high risk of malignant transformation of oral leukoplakia. International Journal of Cancer International du cancer. 2013; 132(4):868–74. Epub 2012/07/12. https://doi.org/10.1002/ijc.27720 PMID: 22782852.

29. Ota N, Ohno J, Seno K, Taniguchi K, Ozeki S. In vitro and in vivo expression of aldehyde dehydrogenase 1 in oral squamous cell carcinoma. International journal of oncology. 2014; 44(2):435–42. https://doi.org/10.3892/ijo.2013.2188 PMID: 24285422.

30. Zhang M, Kumar B, Piao L, Xie X, Schmitt A, Arradaza N, et al. Elevated intrinsic cancer stem cell population in human papillomavirus-associated head and neck squamous cell carcinoma. Cancer. 2014; 120(7):992–1001. https://doi.org/10.1002/cncr.28538 PMID: 24382806.
46. Zhou C, Sun B. The prognostic role of the cancer stem cell marker aldehyde dehydrogenase 1 in head and neck squamous cell carcinomas: a meta-analysis. Oral oncology. 2014; 50(12):1144–8. https://doi.org/10.1016/j.oraloncology.2014.08.018 PMID: 25264224.

47. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer Journal international du cancer. 2017; 14(3):664–70. https://doi.org/10.1002/ijc.30716 PMID: 28369882.

48. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. The New England journal of medicine. 2010; 363(1):24–35. Epub 2010/06/10. https://doi.org/10.1056/NEJMoa0912217 PMID: 20530316.

49. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011; 333(6046):1157–60. Epub 2011/07/30. https://doi.org/10.1126/science.1208130 PMID: 21798893.

50. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011; 333(6046):1154–7. https://doi.org/10.1126/science.1206923 PMID: 21798897.

51. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015; 517(7536):576–82. https://doi.org/10.1038/nature14129 PMID: 25631445.

52. Pysri N, Rampias T, Vermorken JB. The current and future impact of human papillomavirus on treatment of squamous cell carcinoma of the head and neck. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2014; 25(11):2101–15. https://doi.org/10.1093/annonc/mdu265 PMID: 25057165.

53. Linge A, Lohaus F, Lock S, Nowak A, Gudziol V, Valentini C, et al. HPV status, cancer stem cell marker expression, hypoxia gene signatures and tumour volume identify good prognosis subgroups in patients with HNSCC after primary radiochemotherapy: A multicentre retrospective study of the German Cancer Consortium Radiation Oncology Group (DKTK-ROG). Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology. 2016; 121(3):364–73. https://doi.org/10.1016/j.radonc.2016.11.008 PMID: 27913065.

54. Rietbergen MM, Martens-de Kemp SR, Bloemena E, Witte BI, Brink A, Baatenburg de Jong RJ, et al. Cancer stem cell enrichment marker CD98: a prognostic factor for survival in patients with human papillomavirus-positive oropharyngeal cancer. Eur J Cancer. 2014; 50(4):765–73. https://doi.org/10.1016/j.ejca.2013.11.010 PMID: 24315751.