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Alterations in cancer stem-cell marker CD44 expression predict oncologic outcome in soft-tissue sarcomas

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ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form
28 September 2017
Accepted 3 November 2017
Available online xxx

Keywords:
Soft-tissue sarcoma
Cancer stem cells
CD44
ALDH
EGFR
Oncologic outcomes

ABSTRACT

Background: Cancer stem cells (CSCs) have been shown to resist chemotherapy and promote metastasis after cytotoxic therapies. We sought to determine if the expression of CSC markers (aldehyde dehydrogenase [ALDH], CD44, and epidermal growth factor receptor [EGFR]) predicted outcomes in soft-tissue sarcoma (STS) patients.

Methods: We queried an institutional database of 23 STS patients and evaluated immunohistochemical expression of CSC markers ALDH, CD44, and EGFR. The Cancer Genome Atlas (TCGA) was also queried for STS clinical and genomic data. Disease-specific (DSS) and overall survival (OS) were assessed by univariate and Kaplan–Meier analysis.

Results: Of the 23 institutional patients, the majority was female, had high-grade tumors and had extremity tumors. With a median follow-up of 27 months, nine patients (39%) experienced distant recurrence, and four (17%) died of disease. Mean H-scores at diagnosis (±standard error of the mean) for CD44, ALDH1, and EGFR were 169 ± 27, 77 ± 15, and 144 ± 23, respectively. On univariate analysis, there was a trend for increased CD44 score to predict both worse DSS and OS (hazard ratio = 1.01, 95% confidence interval 1-1.02, $P = 0.056$), whereas ALDH and EGFR scores did not. Analysis of 74 TCGA STS cases with complete clinical and genomic data revealed that CD44 copy number alterations predicted worse DSS (9.89 months *versus* 72.5 months, $P = 0.007$) and a trend for worse OS (14.03 months *versus* 38.6 months, $P = 0.12$), whereas ALDH1 and EGFR copy number

Presented in part at the Society for Surgical Oncology 69th Annual Symposium, Boston, MA in March 2016.

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https://doi.org/10.1016/j.jss.2017.11.016

alteration did not. Multivariate analysis of the combined data sets was consistent with worse DSS among patients with higher CD44 expression.

Conclusions: Institutional and national TCGA data show the association of elevated baseline CD44 expression with worse STS outcomes. Further study of CD44 as a possible novel STS biomarker appears indicated.

Introduction

The cancer stem-cell (CSC) hypothesis postulates that a subpopulation of quiescent cells exist within tumors which are resistant to conventional cytotoxic/antiproliferative therapies.[1-3] It is these CSCs which then seed tumor relapse, even in cases of apparent complete response to conventional cytotoxic treatments such as chemotherapy and radiotherapy (RT).[4-6] Cell-surface markers (such as CD24, CD44, and CD133) as well as the intracellular enzyme aldehyde dehydrogenase (ALDH) have been used to identify CSC subpopulations in preclinical and clinical models, and CSC subpopulations have been identified in nearly all human malignancies.[7-9] Moreover, complex molecular and genetic analyses, including lineage tracing in stem cell–transfected reporter mice, have provided high-level/high-impact evidence that CSCs exist and recapitulate key aspects of stem-cell biology, including the capacity for self-renewal, differentiation, and homeostatic control.[4,10-12] As a result of these studies in squamous-cell carcinoma, intestinal adenomas, glioblastoma multiforme, and gastric cancer (among others), a growing body of work is focused on the clinical and biological importance of these cells, including efforts to preferentially target these cells to improve outcomes.[13,14]

CSC markers—including the cell-surface markers CD44 and epidermal growth factor receptor (EGFR) and ALDH—have been analyzed in diverse models.[1,8,15,16] Despite observed variations in the expression of CSC markers across tumors and the models evaluated, these markers have been consistently identified as characteristic of stem-like cells in breast cancer, prostate cancer, and numerous other solid tumors, including soft-tissue sarcomas (STSs).[15,17,18] Furthermore, the levels of $ALDH^{bright}$ cells as well as other CSC markers such as CD44 and EGFR have been observed to predict worse oncologic outcome across the spectrum of solid and hematological malignancies.[19-24] In addition, overexpression of these markers has been correlated with the CSC phenotype as well as resistance to chemotherapy, RT, and epithelial-to-mesenchymal transition.[1,25]

Classically, validation of the CSC phenotype is predicated on functional assessment of stem-like tumor repopulation/tumor initiation behavior, including tumor xenograft formation in immunosuppressed mice, colony outgrowth in non-adherent cell culture conditions, or spheroid formation.[1] In both preclinical models and primary STS specimens, we have previously shown that antiproliferative therapies such as RT enrich for tumorigenic sarcoma CSCs, in particular $ALDH^{bright}$ CSCs.[26-28] Although we were unable to directly evaluate for CSC behavior or function in archived STS specimens, in this study, we sought to determine if we could detect expression of CSC markers in clinical STS specimens and whether expression of these CSC markers predicted oncologic outcomes in patients with primary STS undergoing treatment with curative intent.

Methods and materials

Evaluation of archived clinical sarcoma samples

This study was approved by the Institutional Review Board. Since it was considered no more than a minimal risk, a waiver of consent was obtained. We then identified 23 patients from July 2011 to January 2015 from a prospectively maintained cancer center database who underwent initial diagnostic biopsy at our institution followed by multimodality therapy (including surgical resection) with curative intent and who had sufficient formalin-fixed, paraffin-embedded archived tumor tissue available for the construction of a tissue microarray (TMA).

We then abstracted clinical, pathologic, and treatment data, including age, gender, tumor location, stage at presentation, histologic type, maximal tumor diameter, histologic grade, and tumor depth. Tumor size was analyzed as a continuous variable using maximal tumor dimension from initial pathological evaluation. Tumor sites included extremity (upper at or distal to the shoulder/axilla and lower at or distal to the buttock/groin) and trunk. Retroperitoneal and visral tumors were excluded. Histologic grade was classified using a three-tier system (grade I-III) according to the established criteria.[29]

Histologic diagnosis was assigned by the published criteria of the World Health Organization Classification of Tumors of Soft Tissue and Bone.[29] For the purposes of statistical analysis, we limited our analysis to four histology categories, including liposarcoma, leiomyosarcoma, undifferentiated pleomorphic sarcoma (UPS), and "other" which represented a composite of synovial sarcoma, extraskeletal myxoid chondrosarcoma, solitary fibrous tumor, angiosarcoma, fibromyxoid sarcoma, clear-cell sarcoma, epithelioid sarcoma, primitive neuroectodermal tumor, and sarcoma, not otherwise specified.

The date of recurrent disease was defined either by biopsy or by the radiologic detection of suspicious lesions when no biopsy was performed. Follow-up was counted from the date of diagnosis until the date of death or date of last follow-up. Patients who were free from recurrence or death were censored according to the date of their last follow-up. Distant recurrence-free, disease-specific (DSS), and overall survival (OS) were calculated as described previously.[30,31]

After antigen retrieval and blocking, TMA sections (4 μm) were immunostained using a commercially available purified mouse anti-human ALDH1 antibody (BD Transduction