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The impact of Aldehyde dehydrogenase 1 expression on prognosis for metastatic colon cancer

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ABSTRACT

Background: Cancer stem cells may be associated with tumor progression and prognosis for colon cancer. We hypothesized that expression of Aldehyde dehydrogenase 1 (ALDH1) would increase with tumor progression and be associated with survival.

Methods: Tissue was obtained from resection specimens for isolation of cancer stem cells. In addition, paraffin blocks from resected colon cancers with normal colon, primary tumor, and lymph node and liver metastasis from 2000 to 2010 were identified and stained with ALDH1.

Results: In *in vitro* models (adherent and tumor spheres) ALHD1+ cells grew more efficiently than ALDH1− cells. ALDH1 expression was highest in peritumoral crypt cells (0.137 μm^2, 95% confidence interval [CI] 0.125–0.356) and normal crypts (median 0.091 μm^2, 95% CI 0.064–0.299) followed by lymph node metastasis (median 0.025 μm^2, 95% CI 0–0.131) and the primary cancers (median 0.014 μm^2, 95% CI 0.0123–0.154). Samples were divided into high and low ALDH1 expression. Survival was associated with expression in the primary tumor (9 *versus* 23 mo, $P = 0.0016$) expression but not peritumoral tissue (21 *versus* 20.5 mo, $P = 0.32$), normal colon (19 *versus* 27 mo, $P = 0.289$), or lymph node metastasis (23 *versus* 21 mo, $P = 0.69$). On univariate analysis, ALDH1 expression and grade were associated with survival but ages, number of lymph node metastasis, race, or grade were not associated. On multivariate analysis, only ALDH1 status continued to be associated with survival, odds ratio 4.4, and $P = 0.011$.

Conclusions: ALDH1 is indicative of stemness and is a biomarker marker in colon cancer. Expression did not increase with progression from normal colon to primary tumors and metastasis.

1. Introduction

Personalized medicine, a mantra in cancer treatment, has yet to have a meaningful impact on patients with colorectal cancer. Standard prognostic features such as American Joint Committee on Cancer stage, lymph node ratio, grade, obstruction, perforation, and more recently molecular techniques (Oncotype DX Colon) can classify patients to high or

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low risk regarding recurrence [1]. Unfortunately, many patients at the low-risk group will die from recurrence, whereas high-risk patients treated with adjuvant chemotherapy still succumb to disease. Furthermore, patients with metastatic disease have variable response to chemotherapy. Currently, adjuvant therapy regimens are, for the most part, not individualized in colorectal cancer and regimen selection is often empiric in metastatic disease.

Many postulate that cancer stem cells (CSC) or cells with stem cell characteristics (cancer initiating cells) are associated with tumor growth, maintenance, metastasis, and repopulation as well as prognosis and resistance to therapy [2,3]. This concept was originally postulated by Cohnheim in 1875 and it has been recently more fully investigated [4]. The hypothesis was first tested in the 1990s when investigators found that a few cells within leukemia could, when transplanted into a proper host, reconstitute the neoplasm [5].

CSC are perhaps responsible for chemoresistance and predictive of response to treatment. CSC, like the normal stem cells, divide slowly and thus may evade typical cytotoxic chemotherapy regimens [2]. In addition, activation of the multiple drug resistant transport 1 and developmental pathways (i.e., Wnt and Notch) may also impart chemoresistance [2]. Multiple markers can aid in the identification of colon CSC. The first cell surface markers used for the isolation of colon CSC were CD133, CD44, and CD166 [6–10]. More recent data suggest that Aldehyde dehydrogenase 1 (ALDH1) and Lgr5 may be superior markers in detecting stemness [11,12]. The classic model for accessing stemness is introduction of putative stem cells into an immune compromised mouse with subsequent tumor recapitulation [5]. A surrogate *ex vivo* technique is growth of tumor spheres in three dimensional cultures in the presence of growth factors. These tumor spheres are considered a surrogate marker for stemness as they consistently reproduce neoplasms with a tumor gene expression profile and morphology similar to that of the original cancer when implanted into nonobese diabetic severe combined immuno-deficient mice [11].

ALDH1 has been linked to stemness in many tissues including hematopoietic, mesenchymal, neural, mammary, and prostate [13–15]. The exact function (s) of ALDH1 is unknown, but it is important in retinoic acid biosynthesis, ethanol metabolism, and metabolism of alkylating agents [16]. Activation of retinoic acid signaling may play a role in embryonic development and stem cell maintenance [16]. Increased expression of ALDH1 and other stem cell markers has been associated with colon cancer stage and prognosis [17]. Langan *et al.* [18] assessed a panel of CSC markers (CD29, CD44, ALDH1AI, ALDH1B1, EpCam, and CD166) in 30 patients with various stages of colorectal cancer. The investigators found that increased expression of colon CSC markers was associated with advanced stage, grade, lymph node metastasis, Stage IV, and survival [4]. Similarly, Coco *et al.* [19] found that increased expression of CD133 was associated with recurrence of colorectal cancer and decreased survival. ALDH1 may be a more universal prognostic marker because increased expression predicts decreased survival for multiple cancers including urothelial, nasopharyngeal, breast, pancreatic, and serous ovarian cancers [13,16,20].

In this study, we hypothesized that expression of ALDH1 would increase in metastatic disease compared with normal colon and primary colorectal tumor. Also, ALDH1 levels in primary tumors will be associated with survival. To test this hypothesis, we performed immunohistochemical analysis for ALDH1 on sections derived from paraffin-embedded tissue obtained from patients with blocks from normal, primary colorectal cancer, and lymph node and liver metastasis.

2. Materials and methods

2.1. Study population

Study design and execution were approved by the East Carolina University Institutional Review Board. Patients undergoing surgical resection for colorectal cancer between 2000 and 2010 were identified. There were a total of 726 patients with colorectal cancer, of which we selected only those with Stage IV colon cancer with resection specimens. Paraffin blocks were selected only if specimens from normal colon, primary cancer, and lymph node and liver metastasis were available for analysis. A total of four slides per patient (if sample available and analyzable) were prepared (normal colon, primary cancer, lymph node, and liver). Demographic and survival data were obtained from the Vidant Medical Center tumor registry and patient medical records.

In addition, fresh tissue was obtained for the isolation and culture of epithelial stem cells. Patients undergoing surgical resection for primary colorectal cancer were identified by the staff of North Carolina Tissue Consortium and consented for tissue procurement via an ongoing Institutional Review Board approved trial. Primary normal and neoplastic epithelial cells were procured shortly after resection.

2.2. Processing of fresh specimens

At the time of operative procedure, fresh tissue was procured. Under sterile conditions, both normal and neoplastic tissue was isolated by the pathologist, and both specimens were confirmed by histologic analysis. Specimens were processed by removing the epithelial areas and neoplastic tissues from the adipose tissue, connective tissue, and adjacent blood vessels. Tissue was then minced and digested in media containing insulin (10mcg per mL), hyaluronidase (100 U/mL), and collagenase (200 U/mL). The digested tissue was centrifuged at 300*g* for 5 min and the supernatant was removed. The cells were washed twice with phosphate-buffered saline.

2.3. Cell isolation and culture

CSC were isolated from fresh tissue as previously described [21,22]. Briefly, tumors were processed as mentioned previously and single cell suspensions were prepared after a standardized protocol. After the enzymatic disassociation and washing, cells were filtered through a 40 μm screen and clumps removed [22]. Cells were stained with Epcam to identify the epithelial component. Epcam positive cells labeled with PE-conjugated ALDH1 antibodies (BD Bioscience, St. Joe, CA) were sorted by flow cytometry into ALDH1 positive and ALDH1 negative subfractions. Cells were cultured in either a low adherence or an adherent