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Aldehyde dehydrogenase 1A3 influences breast cancer progression via differential retinoic acid signaling

Paola Marcato[a,b,*], *Cheryl A. Dean*[b], *Rong-Zong Liu*[c], *Krysta M. Coyle*[a], *Moamen Bydoun*[a], *Melissa Wallace*[a], *Derek Clements*[a], *Colin Turner*[d], *Edward G. Mathenge*[d], *Shashi A. Gujar*[b,e], *Carman A. Giacomantonio*[d], *John R. Mackey*[c], *Roseline Godbout*[c], *Patrick W.K. Lee*[a,b,**]

[a]*Department of Pathology, Dalhousie University, Halifax, NS, Canada*
[b]*Department of Microbiology & Immunology, Dalhousie University, Halifax, NS, Canada*
[c]*Department of Oncology, University of Alberta, Edmonton, AB, Canada*
[d]*Department of Surgery, Dalhousie University, Halifax, NS, Canada*
[e]*Strategy and Organizational Performance, IWK Health Centre, Halifax, NS, Canada*

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ABSTRACT

Aldehyde dehydrogenase (ALDH) 1A enzymes produce retinoic acid (RA), a transcription induction molecule. To investigate if ALDH1A1 or ALDH1A3-mediated RA signaling has an active role in breast cancer tumorigenesis, we performed gene expression and tumor xenograft studies. Analysis of breast patient tumors revealed that high levels of ALDH1A3 correlated with expression of RA-inducible genes with retinoic acid response elements (RAREs), poorer patient survival and triple-negative breast cancers. This suggests a potential link between ALDH1A3 expression and RA signaling especially in aggressive and/or triple-negative breast cancers. In MDA-MB-231, MDA-MB-468 and MDA-MB-435 cells, ALDH1A3 and RA increased expression of RA-inducible genes. Interestingly, ALDH1A3 had opposing effects in tumor xenografts, increasing tumor growth and metastasis of MDA-MB-231 and MDA-MB-435 cells, but decreasing tumor growth of MDA-MB-468 cells. Exogenous RA replaced ALDH1A3 in inducing the same opposing tumor growth and metastasis effects, suggesting that ALDH1A3 mediates these effects by promoting RA signaling. Genome expression analysis revealed that ALDH1A3 induced largely divergent gene expression in MDA-MB-231 and MDA-MB-468 cells which likely resulted in the opposing tumor growth effects. Treatment with DNA methylation inhibitor 5-aza-2'deoxycytidine restored uniform RA-inducibility of RARE-containing HOXA1 and MUC4 in MDA-MB-231 and MDA-MB-468

Abbreviations: ALDH, aldehyde dehydrogenase; ATRA, all-trans-retinoic acid; AZA, 5-aza-2'deoxycytidine; CSCs, cancer stem cells; HOXA1, homeobox transcription factor A1; MSP, methylation-specific PCR; MUC4, mucin 4; PPAR/β/δ, peroxisome proliferator activated receptor beta/delta; PPRE, peroxisome proliferator response element; RA, retinoic acid; RAR, retinoic acid receptor; RARβ, retinoic acid receptor beta; RARRES1, retinoic acid receptor responder 1; RXR, retinoid X receptor; RARE, retinoic acid response element.

* *Corresponding author*. Department of Pathology, Dalhousie University, Rm 11F11, 5850 College Street, Halifax, NS, B3H 4R2, Canada. Tel.: +1 902 494 4239.

** *Corresponding author*. Department of Microbiology & Immunology, Dalhousie University, Rm 7P, 5850 College Street, Halifax, NS, B3H 4R2, Canada. Tel.: +1 902 494 8048.

E-mail addresses: paola.marcato@dal.ca (P. Marcato), patrick.lee@dal.ca (P.W.K. Lee).

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cells, suggesting that differences in epigenetic modifications contribute to differential ALDH1A3/RA-induced gene expression in breast cancer. In summary, ALDH1A3 induces differential RA signaling in breast cancer cells which affects the rate of breast cancer progression.

1. Introduction

Aldehyde dehydrogenases (ALDHs) are a family of evolutionarily conserved enzymes (19 isoforms expressed in humans) responsible for oxidizing aldehydes to carboxylic acids (Black and Vasiliou, 2009; Marchitti et al., 2008). Aldehydes are generated by metabolic processes (e.g. lipid perioxidation) and their detoxification is mediated by the ALDHs. A few isoforms (ALDH1A1, ALDH1A2, ALDH1A3) also function in retinoic acid (RA) cell signaling by oxidizing vitamin A metabolite retinal to RA (Black and Vasiliou, 2009; Penzes et al., 1997; Rexer et al., 2001; Zhao et al., 1996).

Once produced in the cytoplasm, RA translocates to the nucleus where it activates nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) and regulates expression of genes with retinoic acid response element (RARE) sequences in their promoters (McGrane, 2007). In 2002, Balmer and Blomhoff compiled a list of over 500 genes whose expression is upregulated or downregulated by RA (Balmer and Blomhoff, 2002). Therefore, RA can modulate a wide variety of biological processes, including differentiation, apoptosis, cell cycle arrest and cell proliferation (Balmer and Blomhoff, 2002; McGrane, 2007; Tang and Gudas, 2011). One proposed mechanism for RA-induced cell proliferation versus cell cycle arrest is predominant activation of alternative nuclear hormone receptors peroxisome proliferator activated receptor beta/delta (PPAR/β/δ) over RARs. Dominant activation of RAR/RXR and transcription of genes with RAREs typically leads to growth suppression. In contrast, dominant activation of PPAR/β/δ/RXR and transcription of genes with peroxisome proliferator response elements (PPREs) leads to cell proliferation (Schug et al., 2007, 2008). An additional alternative mechanism for the differential growth effects of RA is epigenetic silencing of RA-inducible tumor suppressors (Tang and Gudas, 2011). Therefore, the cellular context in which RA signaling occurs has a major effect on the cellular outcomes induced by RA.

Of the RA-producing ALDH1A enzymes, increasing evidence suggests a potential role for ALDH1A1 and ALDH1A3 in cancer. First, increased expression of both ALDH1A1 and ALDH1A3 (but not ALDH1A2) has been reported in Aldefluor-positive-identified cancer stem cell (CSC) populations of breast cancer and melanoma (Ginestier et al., 2007; Luo et al., 2012; Marcato et al., 2011). ALDH1A1 expression is often associated with worse prognosis in cancers, such as breast, prostate and lung (Charafe-Jauffret et al., 2010; Ginestier et al., 2007; Khoury et al., 2012; Li et al., 2010; Li et al., 2012; Morimoto et al., 2009; Neumeister et al., 2010). Additionally, ALDH1A1 expression may contribute to chemotherapeutic resistance in cancer (Moreb et al., 2007; Sladek et al., 2002); however, it has not yet been functionally linked to cancer progression. High ALDH1A3 levels have also been associated with more aggressive forms of breast, glioblastoma, gall bladder cancer and pancreatic cancer (Jia et al., 2013; Mao et al., 2013; Marcato, et al., 2011; Yang et al., 2013; Zhang et al., 2013). Importantly, there is some evidence suggesting that ALDH1A3 may have functional relevance in cancer progression, with ALDH1A3 knockdown in a melanoma cell line resulting in reduced tumor growth (Luo et al., 2012). Similarly, ALDH1A3 knockdown in mesenchymal glioblastoma cells reduced their growth (Mao et al., 2013).

Therefore, we hypothesized that ALDH1A1 and/or ALDH1A3 may have a functional role in breast cancer. Furthermore, since these enzymes are known to produce RA, we wondered if RA signaling in breast cancer is dependent upon expression of either ALDH1A enzyme. Analysis of patient tumor gene expression data revealed that ALDH1A3 expression correlates significantly with expression of RA-inducible genes, poorer survival and triple-negative breast cancers. In breast cancer cell line experiments, expression of RA-inducible genes was dependent upon ALDH1A3 expression and ALDH1A3/RA affected tumor growth and metastasis. Interestingly, depending on the breast cancer cell line, ALDH1A3/RA induced either tumor progression or suppression effects, which at least in part, likely depend upon differential epigenetic modifications of RA-inducible genes. In summary, these findings link expression of ALDH1A3 with differential RA signaling and breast cancer progression.

2. Materials and methods

2.1. Cohort of patients and gene expression microarray analysis

176 treatment-naïve primary breast cancer samples were obtained through the CBCF Tumor Bank and used for gene expression microarray. All 176 frozen dissected tumor samples had been banked by board certified breast cancer pathologists who also performed quality assurance reports (histological and cellular composition quantifications of the tumors) of the clinical tissue samples directly adjacent to (en face of) the banked tissue samples. The clinical tissues were oriented in the formalin fixed paraffin embedded (FFPE) blocks so that when the planes of the clinical tissues are sectioned, they are continuous with the banked samples. The clinical tissues were subjected to routine fixation and hematoxylin and eosin (H&E) staining. Cancer cells, stromal cells and normal

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