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A Rare Eicosanoid Precursor Analogue, Sciadonic Acid (5Z,11Z, 14Z-20:3), Detected In Vivo in Hormone Positive Breast Cancer Tissue

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Abstract

Numerous genetic alterations of HSA 11q13 are found frequently in several cancer types, including breast cancer (BC). The 11q13 locus harbors FADS2 encoding Δ6 desaturation which is not functional in several cancer cell lines, including hormone positive MCF7 BC cells. *In vitro,* the non-functional FADS2 activity unmasks 18:2n-6 elongation to 20:2n-6 and 5-desaturation by FADS1 to yield 5Z,11Z,14Z-20:3 (sciadonic acid) rather than 5Z,8Z,11Z,14Z-20:4 (arachidonic acid). In this pilot study we aimed to determine whether 5,11,14-20:3 appears *in vivo* in hormone positive human BC tissue. Fatty acids were profiled in surgically removed human breast tumor and adjacent normal tissue (n=9). Sciadonic acid was detected in three of nine breast tumor samples and was below detect limits in normal breast tissue. The internal Δ8 double bond of arachidonic acid is required for normal eicosanoid synthesis but is missing in sciadonic acid. This pilot study

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Author Contributions

JTB and KSDK conceived the research; KSDK, JTB, CF and SD designed the research protocol; CF, DS, DAS, JM and SD executed clinical aspects and sampling, HP and JYZ executed the laboratory research; JTB and KSDK contributed new reagents/analytic tools; HP, JYZ, PL, KSDK and JTB analyzed and interpreted the data; KSDK and JTB wrote the first draft; all authors approved the final draft.

Conflict of Interest

All authors declare no conflict of interest.

demonstrates for the first time *in vivo* sciadonic acid in hormone positive BC tissue, warranting a larger survey study to further evaluate its appearance and the functional implications.

Keywords

Breast Cancer; Eicosanoid; Docosanoid; Estrogen; Fatty acid desaturase 2; Cell signaling

INTRODUCTION

Human breast cancer (BC) is the most common cancer among women in US and worldwide, with 2.4 million new cases diagnosed in 2015 [1, 2]. It is the second most common cause of death from cancer in women in US, with estimated deaths of 40,610 in 2017 [2]. The large percentage (~70%) of BC are endocrine-related and ovarian sex hormone estrogen is regarded as both initiator and promoter of BC [3–6]. Another ovarian sex hormone progesterone and its metabolites are also considered to promote BC [7, 8]. The BC expressing estrogen receptors (ER) and/or progesterone receptors (PR) responds to hormone therapy [9].

The human chromosome 11q13 (HSA 11q13) region is well known to be a major cancer hotspot, harboring potential oncogenic driver(s) [10–12]. Various genetic alterations of 11q13 (amplifications, deletions, insertions and translocations) are frequently found events in several cancer types, such as BC, ovarian cancer, cervical cancer, numerous types of squamous cell carcinoma, endocrine tumors, lymphomas and myelomas [13–20]. The biologically active eicosanoids and their metabolites are linked to tumor progression via several mechanisms including dysregulation of cell signaling [21–24]. In humans fatty acid desaturases, FADS1, FADS2, and FADS3 are three enzyme-coding genes localized to human chromosome 11q13 [25] and are required for the biosynthesis of 20 and 22 carbon polyunsaturated fatty acids (PUFA) that are direct cell signaling eicosanoid and docosanoid precursors [26–28]. In several cancer cell lines, including hormone positive MCF7 BC cells, FADS2 encoded Δ6 desaturation is not functional [29–31].

As FADS2 catalyzes the first critical step for the eicosanoid and docosanoid precursor biosynthesis, we hypothesized the depletion/dysregulation of normal eicosanoid and/or docosanoid cell signaling precursor milieu is a potential oncogenic driving event in certain cancer types, including BC. Wild type MCF7 cells have no bioactivity towards the polyunsaturated fatty acids 18:2n-6 and 18:3n-3, whereas transient transfection with FADS2 restores activity [29]. In normal cells, Δ6 desaturation catalyzing 18:2n-6 → 18:3n-6 masks the lower activity competing elongation pathway 18:2n-6 → 20:2n-6. In MCF7 cells, the absence of Δ6 desaturation activity unmasks elongation to 20:2n-6 (11,14-20:2), which then accumulates at modest levels. FADS1 coded Δ5 desaturation then operates on 11,14-20:2 → 5,11,14-20:3, sciadonic acid, which is otherwise below detection limits in normal tissue [29, 32, 33]. The main purpose of the present pilot study is to test for the appearance of the rare (all cis double bonds) 5Z,11Z,14Z-20:3 fatty acid in hormone positive BC tissue *in vivo*, thereby replicating our *in vitro* cell culture findings to provide insight to possible metabolic derangement in fatty acids of hormone positive BC.

Materials and Methods

Study Approvals

The study was approved by The Cornell University Institutional Review Board and The Cayuga Medical Center at Ithaca Institutional Review Board for human participants. A written informed consent was obtained from all nine women participants in this pilot study. All the nine participants were diagnosed with estrogen receptor (ER) and progesterone receptor (PR) positive breast tumors. From each participant surgically removed fresh 50 mg of the breast tumor and 50 mg of the adjacent noncancerous breast tissues were used for fatty acid analysis.

Fatty acid analysis

Breast tumor and adjacent normal breast tissues (Figure 1) were used for fatty acid extraction and analysis. Fatty acid methyl esters (FAME) were prepared using modified one-step method of Garces and Mancha [34] and were analyzed quantitatively using a Hewlett Packard 5890 series II gas chromatograph-flame ionization detector (GC-FID) equipped with a BPX 70 column (25 m, 0.22-mm inner diameter, 0.25 μm film; SGE, Austin, TX) using an equal weight mixture for response factor calibration. The peak structures were positively identified by GC-covalent adduct chemical ionization tandem mass spectrometry (GC-CACI-MS/MS) as previously described [35, 36].

Chemicals

Solvents and reagents for fatty acid extractions were HPLC grade from Sigma-Aldrich (St. Louis, MO, USA) or Burdick & Jackson (Muskegon, MI, USA).

Results and Discussion

Previously, we have shown that ER and PR positive MCF7 breast cancer cells lack the initial FADS2-encoded desaturation activity step in the biosynthesis of long chain polyunsaturated fatty acids from the 18 carbon precursors that are abundant in the human diet. *In vitro* MCF7 cells biosynthesize 5,11,14-20:3 via elongation and FADS1 activity [29]. The 5,11,14-20:3 fatty acid is structurally identical to arachidonic acid (ARA; 20:4n-6; 5,8,11,14-20:4) except it lacks the internal Δ8 double bond required for prostaglandin and leukotriene synthesis, among other eicosanoids. A clear question is whether 5,11,14-20:3 is present *in vivo*, and specifically in ER and PR positive breast cancer tissue. We find 5,11,14-20:3 presence in 3 out of 9 breast tumor samples analyzed (Figure 2, Top). None of the adjacent noncancerous samples showed 5,11,14-20:3 above detection limits (Figure 2, Bottom).

Figure 3A presents the CACI-MS spectrum of 20:3, showing the familiar peaks at *m/z* 374, 321, 289, and 271, corresponding to the $[M+54]^+$, $[MH]^+$, $[M+54-32]^+$, and $[MH+54-32-18]^+$ ions, respectively, characteristic of a 20:3 FAME. Figure 3B is the collisionally activated dissociation spectrum (MS/MS) of $[M+54]^+$ of 5,11,14-20:3, yielding ions at *m/z* 304 and 192 corresponding to the α and ω diagnostic ions, respectively, positively identifying the internal diene double bond structure of this fatty acid. The additional peak at m/z 272 locates the isolated 5-6 double bond.

Dysfunctional endogenous and de novo fatty-acid synthesis has long been recognized as a characteristic of human cancers, including BC [37, 38]. The detection of 5,11,14-20:3 in noncancerous tissues has not been reported, however, it has been detected in mammalian tissues and cells in animals fed 11,14-20:2 or 5,11,14-20:3 [29, 32, 33]. Feeding mice with small quantities of 5,11,14–20:3 significantly replaced the normal eicosanoid precursor ARA in the phosphatidylinositol (PI) fraction [39]. The acyl composition of PI is highly resistant to dietary modifications reflecting PI's role as a major messaging lipid. Replacement of ARA in PI pools by 5,11,14-20:3 may lead to the production of novel secondary messengers [39]. In another mouse study, feeding 5,11,14-20:3 for a 2-week period resulted in 50% reduction in the levels of ARA in hepatic PI fractions. This reduction in the ARA content may have an influence on eicosanoid signaling since eicosanoid synthesis has long been known to be related to the abundance of ARA. In the same study 5,11,14-20:3 was extensively incorporated into hepatic phosphatidylinositol bisphosphate (PIP2), a precursor of second messengers. Similarly, 5,11,14-20:3 reduced the ARA content of the PI fraction in the HepG2 cells which may have influence on PI-originating bioactive lipids [40, 41]. The phosphoinositide signaling pathway is the most frequently altered in BC and alterations in this pathway are associated with resistance to hormone therapy [42, 43]. PhospholipaseA2 has poor affinity for 5,11,14-20:3, suggesting that 5,11,14-20:3 accumulate in membrane phospholipid (PL) pools even with moderate availability [44].

Swiss 3T3 cells, when cultured with 5,11,14-20:3 and stimulated using 100 nM of bombesin, produced 1-stearoyl-2-sciadonoyl-glycerol. 1-Stearoyl-2-sciadonoyl-glycerol was found to activate diacylglycerol-protein kinase C (PKC) signaling [45]. Similarly, in NG108-15 cells 1-stearoyl-2-sciadonoyl-glycerol (G) induced monoacylglycerol signaling, through a CBI receptor-dependent mechanism [46]. Topical application of 5,11,14-20:3 to mouse ear reduced ARA induced edema [47]. In colon cancer and a few other cell lines, exogenous ARA treatment caused apoptosis, suggestive of unesterified ARA signals induction of apoptosis [48–50].

Several studies have reported a positive correlation between amplification/overexpression of certain genes on 11q13 and estrogen receptor (ER) positive status [51–53]. Microdissection of tissue sections from same patients (15 of 15) showed loss of heterozygosity (LOH) of the identical allele at 11q13 in early stage and invasive BC, suggesting that important tumor suppressor gene(s) at 11q13 are associated with the development of BC [54] and micro-cell mediated transfer of HSA11 into MCF7 cells reduced tumorigenicity [55]. FADS2 is a multifunctional enzyme with known substrate specificities for 16, 18, 20, 22 and 24 carbon fatty acids [26]. Functional loss of FADS2 affects saturated, monounsaturated and polyunsaturated fatty acid levels, directly influencing eicosanoid and docosanoid pathways and signaling. FADS2 has not been carefully considered as an 11q13 candidate to be a tumor suppressor in neoplastic disorders. The dysregulation of ARA-based eicosanoid signaling has been implicated in the development and progression of BC [56, 57].

Conventional electrospray-MS based lipidomics does not discern fatty acid isomers (positional, geometric, or branched chain) and therefore 5,11,14-20:3 would only be detected as a mixture of 20:3 isomers, normally dominated by 20:3n-6 (dihomo-gamma-linolenic acid). GC-CACI-MS/MS method, developed in our lab, identifies the position and

geometry of double bonds directly in FAME. Alternative online methods for double bond localization by electrospray MS are in progress but have not been comprehensively demonstrated for polyunsaturates of complex double bond structure[58].

Our ability to collect samples limited this pilot study. Microdissection of tumor tissues to acquire maximum tumor load was not carried out because at least 50 mg of tissue was required for fatty acid analysis. As the microdissections were not carried out, piecing of tumor versus necrotic/apoptotic and normal tissue was not possible. In our study the ARA peak was found to be several fold larger in the tumor breast tissue compared to the normal breast tissue (Figure 2). We normalized the peaks to 20:2n-6 FA, the immediate precursor of 5,11,14-20:3. It is well known that the tumor microenvironment contributes to tumor heterogeneity. The cellular environment of a developing tumor consists of a scaffold of extracellular matrix composed of a dynamic variety of non-cancerous immune and stromal cells required for tumor growth, invasion and metastasis [59]. Several studies have indicated that unesterified arachidonic acid (AA) in cells can serve as a second messenger and yields a proapoptotic signal [48, 60, 61]. Tumor Necrosis Factor-induced ARA release in tumor cells has been reported [62] and a recent study has shown accumulation of ARA on the outer edge of the colorectal cancer [63]. Normal as well as necrotic/apoptotic tissue may have dominated the tumor sample in the six samples which we did not find sciadonic acid. Future survey studies with larger sample size and maximum tumor load samples are warranted to delineate the appearance of 5,11,14-20:3 in breast tumor tissues. Existing knowledge about the upstream synthesis pathway for 5,11,14-20:3 points to a genetic aberration at 11q13 and a specific switch to an alternative pathway for its synthesis. Implication for deranged signaling due to interference with ARA derived normal signals suggests that the presence of this unusual fatty acid in membranes may have functional implications for cell-to-cell signaling.

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Highlights

- Sciadonic acid, 5Z,11Z,14Z-20:3, is an arachidonic acid analogue in cells missing 6-desaturation.
- For the first time, sciadonic acid is detected in human breast cancer but not in adjacent healthy tissue.
- Sciadonic acid is not a substrate for prostaglandins and its substitution for arachidonic acid may affect cell-cell signaling.

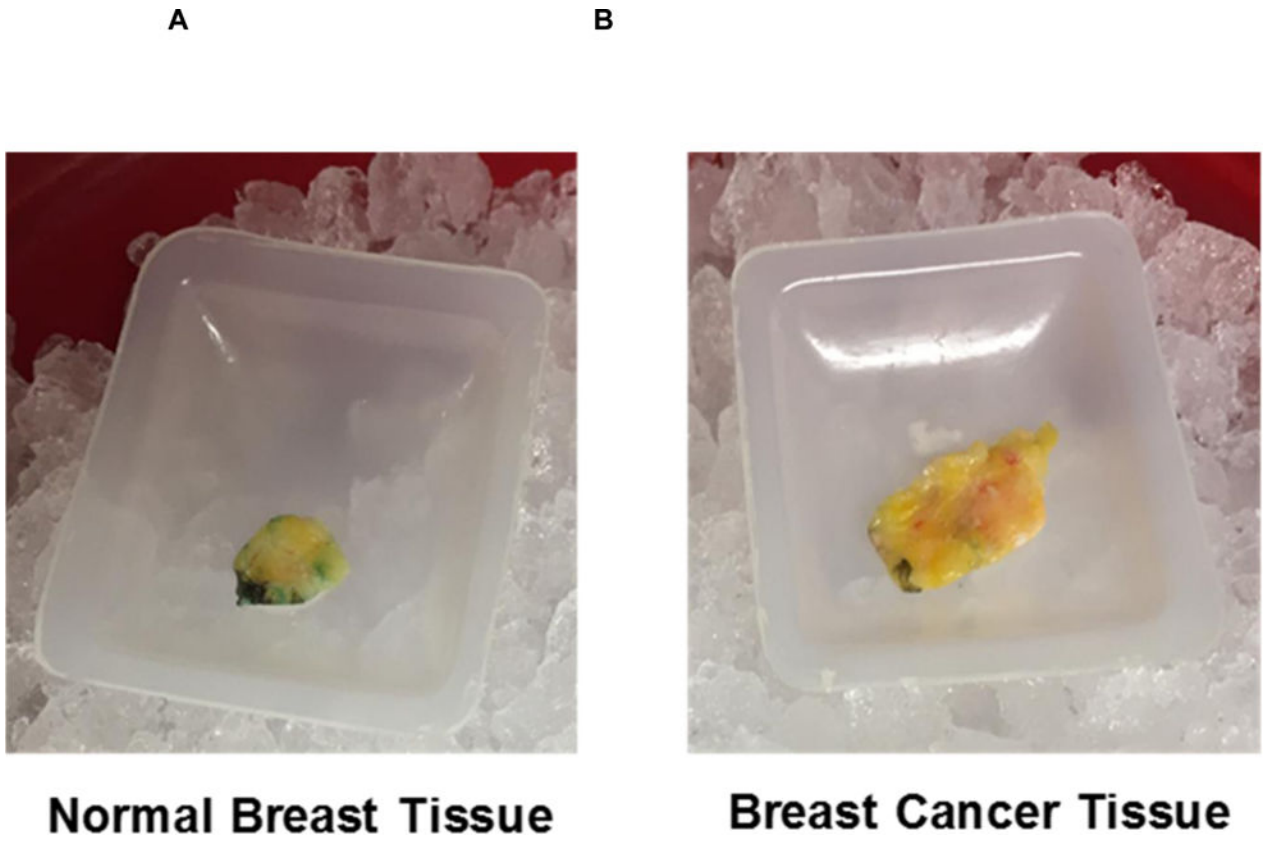

Figure 1.
Surgically removed human breast tissue samples. A) Normal breast tissue B) Breast cancer tissue. Residual blue dye is present in some samples.

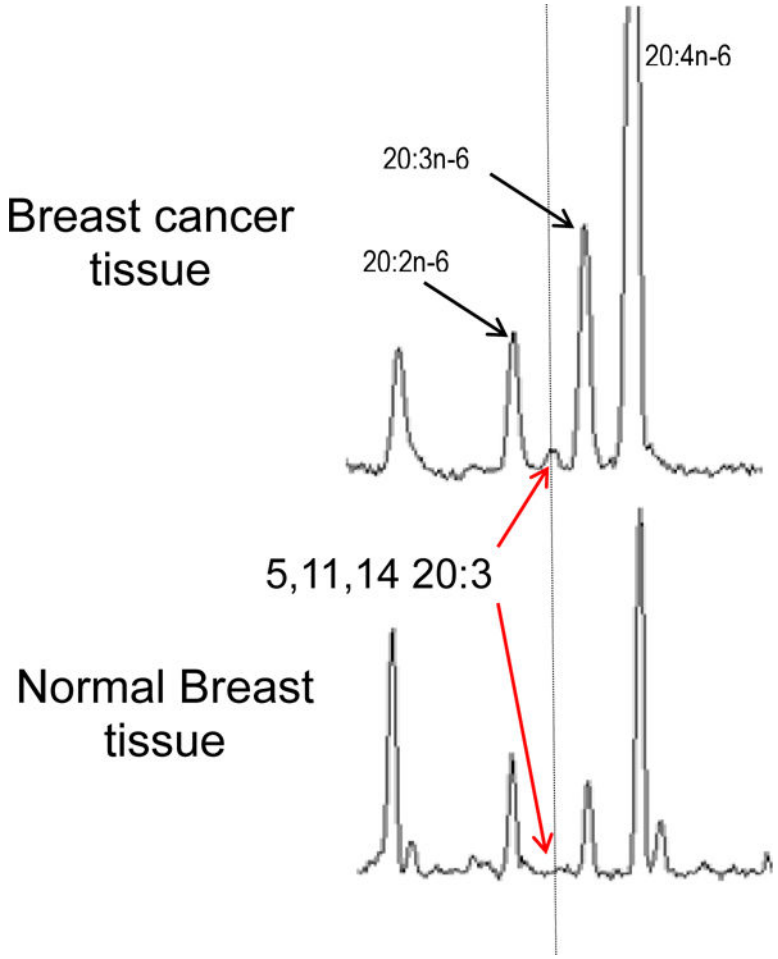

Figure 2.

Gas chromatography results, normalized to the peak for 20:2n-6, from breast cancer and normal breast tissues. Top) Breast cancer tissue. Unusual 5,11,14-20:3 sciadonic acid detected in BC tissue at about 10% (area percent) of the precursor 20:2. 5,11,14-20:3 was found in three of nine samples. Bottom) Normal breast tissue. 5,11,14-20:3 is below detection limits.

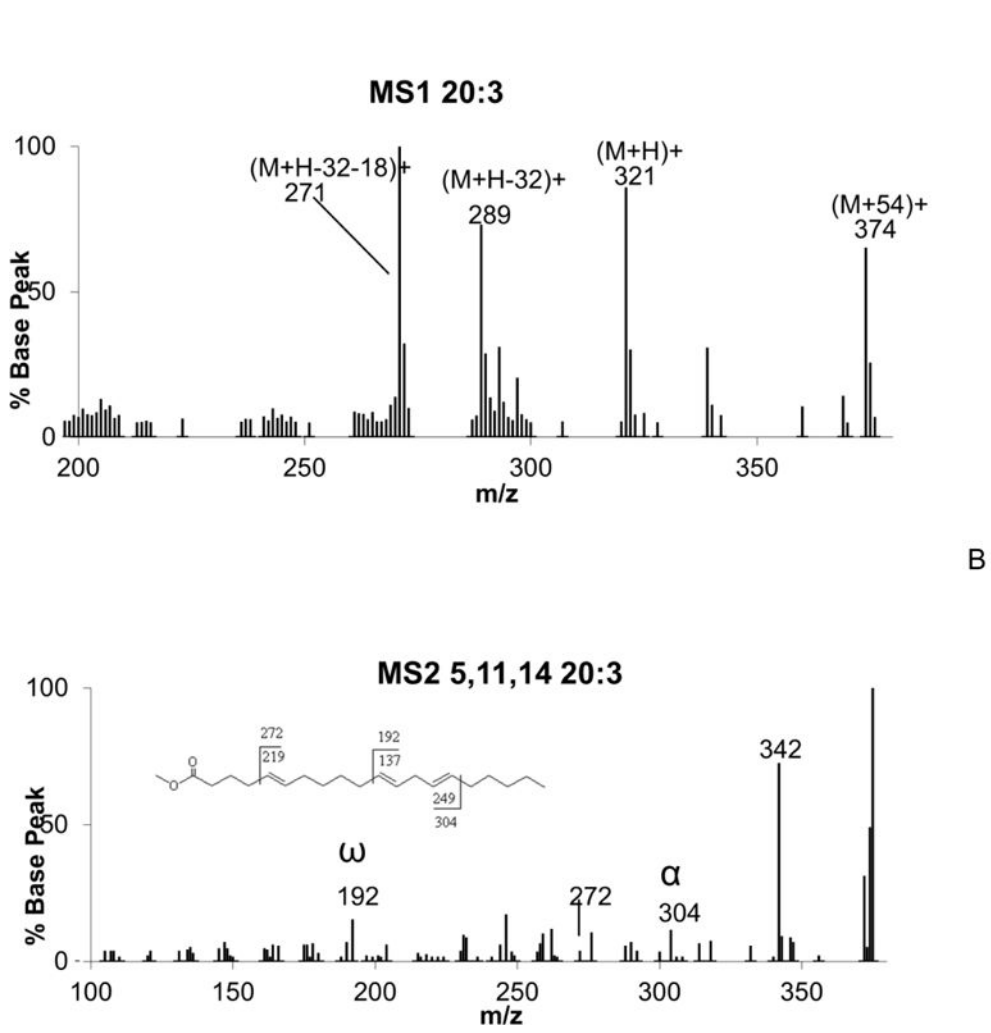

Figure 3.

CACI-MS1 and CACI-MS2 spectra of 20:3 FAME. A) CACI-MS1 spectrum showing m/z 374, 321, 289, and 271 characteristic ions of 20:3 FAME. B) CACI-MS/MS spectrum of the m/z 374 ion positively identifying 5,11,14-20:3 based on the detection of diagnostic ions m/z 304 and 192 for the diene and m/z 272 vinylic to the isolated double bond, for the peak at the expected chromatographic retention time.