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Research Article

Immunohistochemical Coexpression of Androgen Receptor and Its Pioneering Factor Forkhead Box Protein A1 in Salivary Duct Carcinoma

Kouhei Sakurai[1*], Koichiro Wasano[2], Taiji Kawasaki[2], Kimihide Kusafuka[3], Makoto Urano[4], Asako Okabe[4], Mitsuru Nakagawa[4], Makoto Kuroda[4], Masao Kasahara[5], Kazuhiro Tashiro[5], Naoki Kondo[1], Mina Ikeda[1], Ayaka Nakajima[1], Yoshihiro Imaeda[1], Kazuya Shiogama[6], Yasuyoshi Mizutani[6], Takanori Onouchi[6], Masaru Hikichi[7], Ryoichi Shiroki[7], Teiko Sato[8], Yutaka Tsutsumi[6], Ken-ichi Inada[1]

[1]Department of Diagnostic Pathology II, Fujita Health University, School of Medicine, Aichi, Japan

[2]Department of Otolaryngology, Japanese Red Cross Shizuoka Hospital, Shizuoka, Japan

[3]Pathology Division, Shizuoka Cancer Center, Shizuoka, Japan

[4]Department of Diagnostic Pathology I, Fujita Health University, School of Medicine, Aichi, Japan

[5]Department of Diagnostic Pathology, Japanese Red Cross Shizuoka Hospital, Shizuoka, Japan

[6]Department of Pathology, Fujita Health University, School of Medicine, Aichi, Japan

[7]Department of Urology, Fujita Health University, School of Medicine, Aichi, Japan

[8]Department of Diagnostic Pathology, Keiyu Hospital, Kanagawa, Japan

[*]**Corresponding author:** Kouhei Sakurai, Department of Diagnostic Pathology II, Fujita Health University School of Medicine, 3-6-10, Otobashi, Nakagawa-Ku, Nagoya city, Aichi, Japan, 454-8509, Tel: +81 52 323 5957; E-mail: ks3wn@fujita-hu.ac.jp

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Abstract

Androgen Receptor (AR) is a nuclear receptor that regulates the transcription of target genes together with several cofactors. The growth of AR-positive salivary duct carcinoma (SDC) is reported to be androgen dependent. Although AR is considered as a therapeutic target and androgen deprivation therapy (ADT) might be effective in SDC based on its effect on other AR-expressing cancers, such as prostate cancer and apocrine carcinoma of the breast, its detailed molecular gene signature in AR-positive SDC is unclear. To gain insight into the molecular pathogenesis of SDC, we compared the morphology and Immunoprofiles of SDC (n=15), prostate cancer (n=37), and apocrine carcinoma of the breast (n=11) to reveal their shared features. Morphologically, SDC and apocrine carcinoma of the breast shared apocrine-like features. Strikingly, Immunoprofiling revealed that AR and Forkhead Box Protein A1 (FOXA1), a pioneering factor of AR in prostate cancer and apocrine carcinoma of the breast, were concomitantly expressed in SDC (positive; 14 cases, focally positive; 1 case), which indicates that AR-regulated pathways might be active in SDC cells, thus providing evidence for AR-targeted therapy. Together with the apocrine-like microscopic morphology and AR expression, FOXA1 could be used as a diagnostic marker for SDC. Our study suggests morphological and molecular features among SDC and other AR-expressing cancers and helps to understand the molecular pathogenesis of AR+/FOXA1+ SDC.

Introduction

Androgen receptor (AR), a DNA-binding transcriptional factor, is mainly expressed in prostate epithelium and plays critical roles in normal prostate development and prostate cancer progression [1]. AR activation by androgen regulates the transcription of its target genes, such as *kallikrein-related peptidase 3 (KLK3)* in concert with other transcriptional factors such as forkhead box protein A1 (FOXA1) [2-5]. Androgen Deprivation Therapy (ADT), which involves the inhibition of AR activity and/or reduction the amount of its ligand, androgen, is effective in treating prostate cancer [1]. It is clinically relevant and critical to elucidate gene

expression driven by AR-related pathways.

AR is expressed in other cancers as well, including apocrine carcinoma of the breast, which is an additional subgroup of breast carcinoma [6,7]. It is characterized by estrogen receptor negative and AR positive expression. We previously defined apocrine carcinoma of the breast as an estrogen receptor- / progesteron receptor- / AR+ invasive breast ductal carcinoma with apocrine-like microscopic appearance [8]. Signal transduction driven by AR and its associating factor, FOXA1, was previously shown to be important for the growth *in vitro* and *in viv*o [7], similar to that observed in prostate cancer. Recently, ADT is reported to be effective in the treatment like prostate cancer [9,10].

Salivary duct carcinoma (SDC), most cases of which express AR, is a relatively rare salivary cancer [11-13]. SDC is defined by the World Health Organization (WHO) as an aggressive adenocarcinoma that resembles high-grade breast ductal carcinoma (International Classification of Diseases for Oncology (ICD-O) code, 8500/3). A treatment other than surgical resection needs to be developed due to its aggressive clinical outcomes such as high incidence of metastasis [13]. The growth of SDC is reported to be AR dependent like prostate cancer and apocrine carcinoma of the breast [14]. Furthermore recent paper reported that the molecular phenotype of SDC is similar to that of apocrine carcinoma of the breast [15]. Considering that ADT in SDC patients was shown to completely diminish tumor growth and metastasis in some cases [16], SDC might share the phenotypes with other AR-expressing cancers. However, the activation status of the AR-related pathway in SDC remains to be elucidated.

Features that SDC might share with other AR-expressing cancers would be expected to reveal useful diagnostic markers and common therapeutic targets. In this study, we analyzed Immunoprofiles of SDC, apocrine carcinoma of the breast and prostate cancer. Strikingly AR and FOXA1 were concomitantly expressed in SDC and the other two cancers, suggesting that AR-related pathways might be active in SDC. These results contribute to our understanding of the molecular pathogenesis of SDC and suggest novel therapeutic strategies targeting AR-related pathways.

Materials and Methods

Ethical Issue

This study was approved by the Ethics Committee for Clinical Studies of Fujita Health University, Aichi, Japan (#HM15-538), Japanese Red Cross Shizuoka Hospital, Shizuoka, Japan and Keiyu Hospital, Kanagawa, Japan. Written informed consent was obtained from all patients.

Human materials

In this study, we regarded AR-positive salivary malignancies with breast ductal cancer-like structures or granular eosinophilic cytoplasm of tumor cells with round and swollen nuclei as SDC cases. All the specimens surgically resected or biopsied were fixed in 10% formalin and embedded in paraffin wax. Paraffin sections of 4 μm thickness were mounted onto the aminosilane-coated glass slides. Hematoxylin and eosin (H&E) staining was performed for evaluating histological features. The patient information is summarized in Supplementary (Table 1).

Immunohistochemical Staining and Scoring

4 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohol. Endogenous peroxidase activity was inhibited by immersion in 0.3% H2O2/methanol solution, and antigen retrieval was performed in the conditions as described in Table 1 in a pressure cooker (Delicio 6L; T-FAL, Rumily, France) for 10 min. Then, the sections were incubated for 12 hr at room temperature with each antibody. The primary antibodies used in Immunohistochemical staining are: anti-AR (AR441, M3562, Dako), anti-FOXA1 (ab23748, Abcam), anti-Ki-67 (MIB1, M7240, Dako), anti-HER2 (413421, Nichirei), anti-GCDFP-15 (23A3, M3638, Dako), and anti-PSA (A0562, Dako). After the incubation, the sections were washed thoroughly in phosphate-buffered solution (PBS). For AR, FOXA1, Ki-67, HER2, GCDFP15 stainings, they were incubated with Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Tokyo, Japan) as secondary antibody for 30 min at room temperature. For PSA staining, they were incubated with Rabbit/HRP (dilution, 1/100) (P0448) (Dako, Denmark) as secondary antibody for 30 min at room temperature.

Antibody against	Source		Dilution	Solution for heat-induced antigen Retrieval	Secondary Antibody
AR	Dako	AR441	1/100	1 mM EDTA, pH 8	Amino Acid Polymer Technique
FOXA1	Abcam	ab23738	1/100	10 mM citrate buffer, pH 7	Amino Acid Polymer Technique
Ki-67	Dako	MIB1	1/100	1 mM EDTA, pH 8	Amino Acid Polymer Technique
HER2	Nichirei	413421	1/2000	10 mM citrate buffer, pH 6	Amino Acid Polymer Technique
GCDFP-15	Dako	23A3	1/200	10 mM citrate buffer, pH 6	Amino Acid Polymer Technique
PSA	Dako	A0562	1/1500	1 mM EDTA, pH 8	Polyclonal goat anti-rabbit immunoglobulin/HRP

Table 1: The antibody list and the experimental conditions for Immunohistochemical staining.

Finally, immune-complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% 3,3'-diaminobenzine tetrachloride (DAB). Nuclear counterstaining was carried out with Mayer's hematoxylin. The antibodies and the experimental conditions are summarized in Table 1.

The judging criteria of the Immunostaining are as follows. "positive : when more than 10% of the tumor cells showed positivity", "focally positive : when 1-10% of the tumor cells showed positivity", "negative : when less than 1% of the tumor cells showed positivity". The Ki-67 labeling index was expressed as the percentage of the number of positive tumor cells. For HER2 scoring, the positivity was divided into four categories as follows. "3+: strongly expressed along the cell membrane", "2+: moderately expressed", "1+: weakly expressed" and "0: no staining". 2 pathologists (K. Sakurai, and K.I.) independently evaluated each staining.

Results

The cellular morphology of SDC is similar to that of apocrine carcinoma of the breast

We collected salivary tumor cases pathologically diagnosed as SDC (n=15) (See "Human materials" in Materials and Methods section for the criteria). Histologically, SDC frequently exhibited an intraductal growth pattern with comedo necrosis, a representative feature of breast ductal carcinoma (Figure 1A, upper left). Of note, in not all, but some cases, SDC showed cytoplasmic protrusion (Figure 1A, upper center). At single cell level, SDC cells had granular eosinophilic cytoplasm with round and swollen nuclei (Figure 1A, upper right). As previously reported [17,18]. These cytoplasmic features were characteristic of apocrine secretion and apocrine carcinoma of the beast showed a similar morphology (Figure 1B, lower left, center and right).

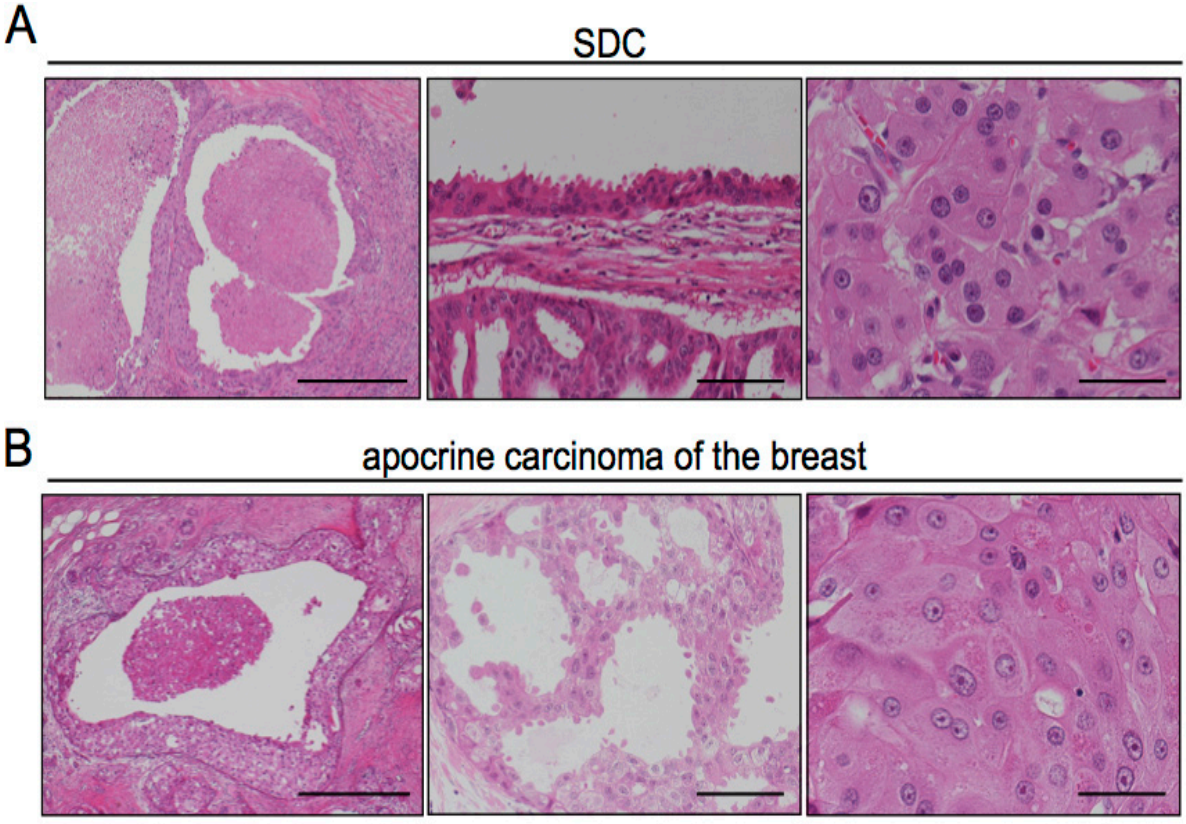

Figure 1: Morphological features of salivary duct carcinoma (SDC) and apocrine carcinoma of the breast. (A) Hematoxylin and eosin (H & E) images of a representative SDC specimen (left, case 4; center, case 13; right, case 10). Scale bars indicate 500 μm (left), 100μm (center) and 50 μm (right). (B) H & E images of a representative apocrine carcinoma of the breast (left, case 11; center, case 2; right, case 6).The rest as in Figure 1A.

AR and FOXA1 are concomitantly expressed in SDC, apocrine carcinoma of the breast and prostate cancer.

Based on shared morphological features as well as previous reports demonstrating AR expression in both SDC and apocrine carcinoma of the breast [8,13], we hypothesized that these cancers might also exhibit similar molecular profiles. We performed im-

munehistochemical staining of several proteins involved in breast cancer, such as human epidermal growth factor receptor 2 (HER2) [8] and gross cystic disease fluid protein 15 (GCDFP-15) [8]. For comparison, we also analyzed prostate cancer (AR-expressing cancer) specimens (n=37) for prostate cancer-related proteins, such as prostate specific antigen (PSA) [19], although the morphology is distinct from those of SDC and apocrine carcinoma of the breast. The protein expression profiles of all cases are summarized in Fig. 2A and Supplementary Table 1 with related clinical information. As seen in Figure 2A and B, AR was expressed in all cases examined, in agreement with previous reports [12,13]. Surprisingly, FOXA1, a pioneering factor of AR in apocrine carcinoma of the breast [7] and prostate cancer [7, 20] cells, was expressed in our 15 SDC cases (positive; 14 cases, focally positive; 1 case) as well. FOXA1 is called "pioneering factor" because it opens the chromatin to recruit AR and enhances AR-mediated transcriptional pathways to induce cellular growth in apocrine carcinoma of the breast and prostate cancer cells [3,7]. Therefore increased FOXA1 expression suggests the activation of AR-driven pathways in SDC cells.

List of the SDC, apocrine carcinoma of the breast and prostate cancer cases Case no, Age, Sex, Ki-67 index (%), HER2 scoring (3+, 2+, 1+, 0), histological type (de novo type, SDC ex pleomorphic adenoma (P.A.) type), Gleason score (sum of the pattern number of the primary and secondary Gleason patterns) are shown.

Our analyses also showed that HER2 and GCDFP-15 were expressed in some, but not all, SDC cases (Figure 2A , B). Interestingly, PSA, whose expression depends on AR [19] in prostate cancer, was expressed at very low levels or was negative in SDC and apocrine carcinoma of the breast specimens despite positive AR expression in these cancers. These findings demonstrated the existence of similarities as well as distinct features among SDC, apocrine carcinoma of the breast, and prostate cancer. One of the most featured distinct characters was Ki-67, which was much higher in SDC than the other two types (Figure 2 A, B).

Strict coexpression of AR and FOXA1 in SDC

Some SDCs arise as the malignant component of a pleomorphic adenoma (carcinoma ex pleomorphic adenoma) [11]. In similar cases included in our study, AR and FOXA1 were specifically expressed only within the SDC but not the pleomorphic adenoma component (Figure 3A). Furthermore, the expression of both AR and FOXA1 were increased within the SDC components in comparison to normal areas (Figure 3B). These results indicate the strict coexpression of AR and FOXA1. Based on our findings herein, FOXA1 immunostaining should be useful for the pathological diagnosis as well as AR expression and the apocrine-like microscopic appearance.

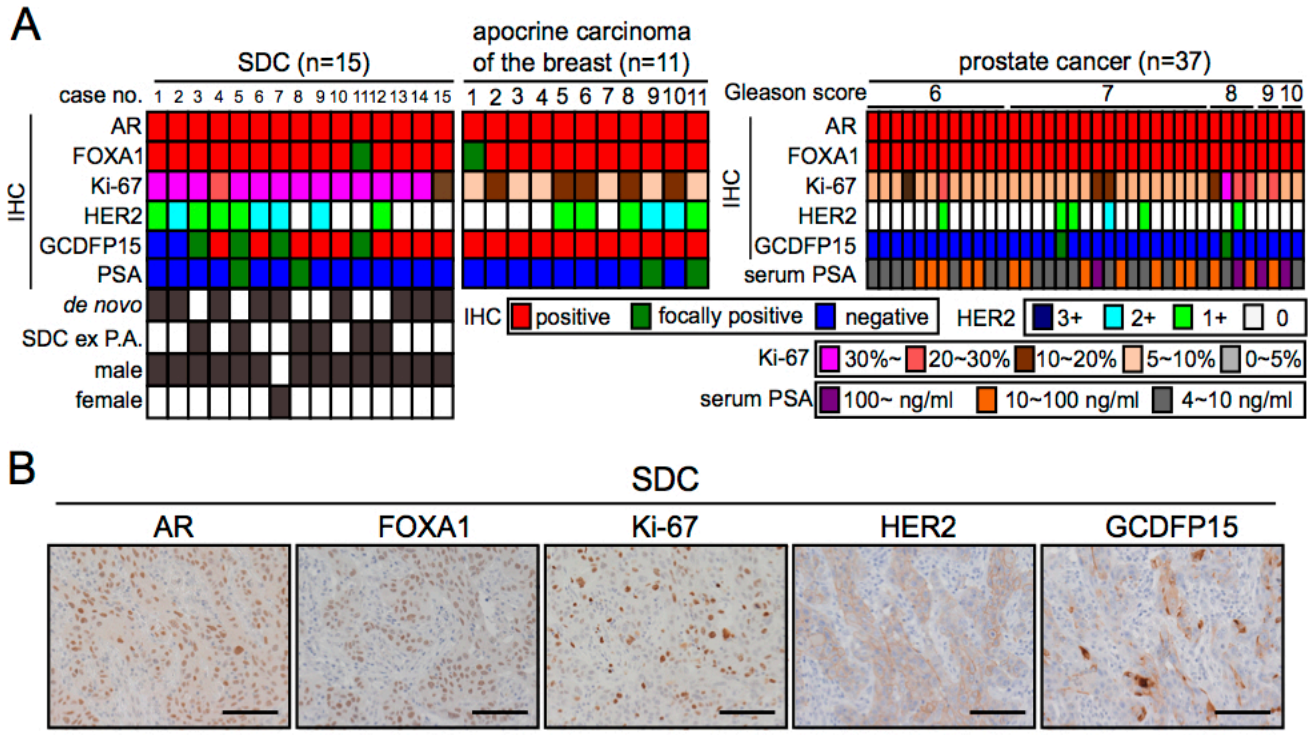

Figure 2: Androgen receptor and forkhead box protein A1 (FOXA1) are expressed in SDC. (A) Summary of the Immunohistochemical staining (IHC) with case numbers and clinical information of specimens from SDC, apocrine carcinoma of the breast, and prostate cancer cases. In prostate cancer cases, the Gleason score and serum total prostate-specific antigen (PSA, ng/ml) are indicated. Case no. of prostate cancer are shown in Supplementary

Table 1. The judging criteria are described in Materials and Methods. (B) The representative images of AR, FOXA1, Ki-67, HER2 and GCDFP15 in SDC (B, case 6). Scale bars indicate 100 μm.

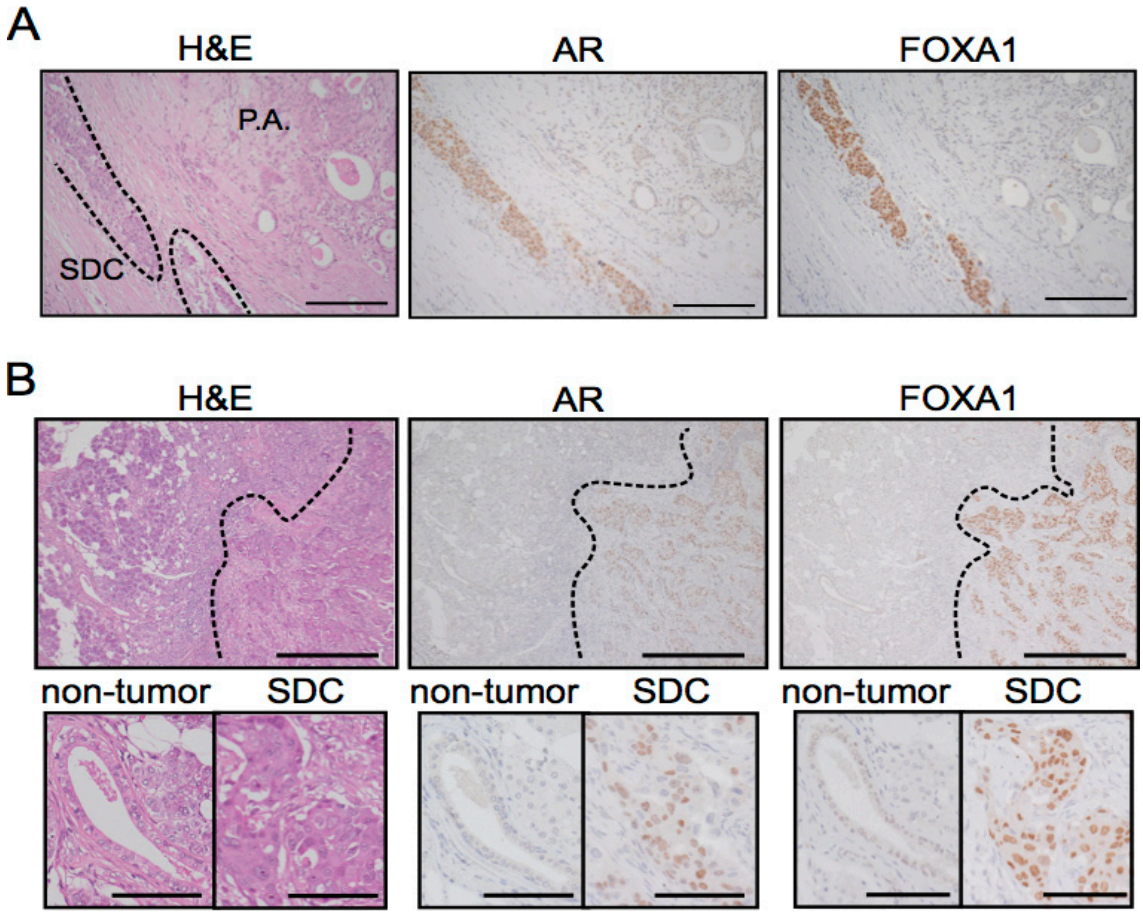

Figure 3: AR and FOXA1 are concomitantly expressed in SDC. (A) H & E and Immunohistochemical staining for AR and FOXA1 in SDC ex pleomorphic adenoma (case 5). The dotted line indicates the border between SDC and pleomorphic adenoma (P.A.). Scale bar indicates 200 μm. (B) H & E image and Immunohistochemical staining for AR and FOXA1 in SDC (case 12). The dotted line indicates the border between SDC and adjacent normal tissue. Scale bar indicates 500 μm (upper panel) and 100 μm (lower panel).

Discussion

This study is a first report demonstrating that AR and FOXA1 are immunohistochemically expressed in SDC and FOXA1 could be a potential diagnostic marker. Although almost all SDC cases tested in this study expressed both AR and FOXA1, we could not rule out the possibility of SDC cases that might not express either or both factors. Analysis of expression of these factors in a larger cohort that will include SDC variants reported by our group (SDC with rhabdoid features) [21] and other groups (e.g., invasive micro papillary variant) [11] is warranted. Furthermore, a study of salivary cancers suggested that AR was not specific for SDC [22]. The assessment should expand to other types of AR-positive salivary tumors.

FOXA1 is a member of the forkhead DNA-binding protein family and opens the chromatin to recruit nuclear receptors, such as ER and AR, acting as a pioneering factor [20]. Robinson et al. showed that FOXA1 establishes the specific gene expression signatures called “molecular apocrine” and induces the cellular growth in apocrine carcinoma of the breast-derived MDA-MB453 cells. Similar to that demonstrated in apocrine carcinoma of the breast, FOXA1 enhances AR-mediated transcriptional pathways and sustains the cellular growth in prostate cancer cells [4,5]. Paradoxically, FOXA1 does not simply act as an oncogenic pioneering factor in prostate cancer. Jin et al. demonstrated that excess FOXA1 leads to excessive opening of the chromatin that dilutes AR binding across the genome [3]. As a result, FOXA1 indirectly inhibits specific AR binding events. Interestingly, they also showed that FOXA1 knockdown decreases cellular proliferation while inducing cellular invasion and metastasis [23].

From our result in this study, FOXA1 expression indicates that AR-related pathways are activated in SDC and AR-targeted therapy such as ADT might be a promising strategy for SDC treatment. However, we emphasize the dual functions (oncogenic and tumor-suppressive) of FOXA1. It is necessary to analyze the transcriptome established by FOXA1 expression during SDC progression to understand the molecular action. Genome-wide study in SDC [15] will help to understand the detail molecular mechanisms.

In summary, FOXA1 could be used as novel diagnostic marker for SDC and AR-related pathways might be active in SDC. Our comparative approach using different cancers allows for a better understanding of similar and dissimilar phenotypes that will help develop novel therapeutic strategies against not only SDC but also a wide range of AR-positive malignancies.

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References

1. Karantanos T, Corn PG, Thompson TC (2013) Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. Oncogene 32: 5501-5511.
2. Sakurai K, Reon BJ, Anaya J, Dutta a (2015) The lncRNA DRAIC/PCAT29 Locus Constitutes a Tumor-Suppressive Nexus. Mol Cancer Res 13: 828-838.
3. Jin HJ, Zhao JC, Wu L, Kim J, Yu J (2014) Cooperativity and equilibrium with FOXA1 define the androgen receptor transcriptional program. Nat Commun 5: 3972.
4. Gerhardt J, Montani M, Wild P, Beer M, Huber F, et al. (2012) FOXA1 promotes tumor progression in prostate cancer and represents a novel hallmark of castration-resistant prostate cancer. Am J Pathol 180: 848-861.
5. Robinson JLL, Hickey TE, Warren AY, Vowler SL, Carroll T, et al. (2014) Elevated levels of FOXA1 facilitate androgen receptor chromatin binding resulting in a CRPC-like phenotype. Oncogene 33: 5666-5674.
6. Farmer P, Bonnefoi H, Becette V, Tubiana-hulin M, Fumoleau P, et al. (2005) Identification of molecular apocrine breast tumours by microarray analysis 24: 4660-4671.
7. Robinson JLL, MacArthur S, Ross-Innes CS, Tilley WD, Neal DE, et al. (2011) Androgen receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1. EMBO J 30: 3019-3027.
8. Tsutsumi Y (2012) Apocrine Carcinoma as Triple-negative Breast Cancer: Novel Definition of Apocrine-type Carcinoma as Estrogen/Progesterone Receptor-negative and Androgen Receptor-positive Invasive Ductal Carcinoma. Jpn J Clin Oncol 42: 375-386.
9. Arce-Salinas C, Riesco-Martinez MC, Hanna W, Bedard P, Warner E (2014) Complete Response of Metastatic Androgen Receptor-Positive Breast Cancer to Bicalutamide: Case Report and Review of the Literature. J Clin Oncol 32: 2-5.
10. Jongen L, Paridaens R, Floris G, Wildiers H, Neven P (2016) Androgen deprivation by adrenal suppression using low-dose hydrocortisone for the treatment of breast carcinoma with apocrine features: a case report illustrating this new paradigm. Breast Cancer Res Treat 155: 603-607.
11. Nagao T, Gaffey TA, Visscher DW, Kay PA, Minato H, et al. (2004) Invasive micropapillary salivary duct carcinoma: a distinct histologic variant with biologic significance. Am J Surg Pathol 28: 319-26.
12. Masubuchi T, Tada Y, Maruya SI, Osamura Y, Kamata SE, et al. (2014) Clinicopathological significance of androgen receptor, HER2, Ki-67 and EGFR expressions in salivary duct carcinoma. Int J Clin Oncol 20: 1-10.
13. Otsuka K, Imanishi Y, Tada Y, Kawakita D, Kano S, et al. (2016) Clinical Outcomes and Prognostic Factors for Salivary Duct Carcinoma: A Multi-Institutional Analysis of 141 Patients. Ann Surg Oncol 23: 2038-2045.
14. Mitani Y, Rao PH, Maity SN, Lee YC, Ferrarotto R, et al. (2014) Alterations associated with androgen receptor gene activation in salivary duct carcinoma of both sexes: Potential therapeutic ramifications. Clin cancer Res 20: 6570-6581.
15. Dalin MG, Desrichard A, Katabi N, Makarov V, Walsh LA, et al. (2016) Comprehensive molecular characterization of salivary duct carcinoma reveals actionable targets and similarity to apocrine breast cancer. Clin Cancer Res 22: 4623-4633.
16. Yamamoto N, Minami S, Fujii M (2014) Clinicopathologic study of salivary duct carcinoma and the efficacy of androgen deprivation therapy. Am J Otolaryngol 35: 731-735.
17. Chiosea SI, Williams L, Griffith CC, Thompson LDR, Weinreb I, et al. (2015) Molecular Characterization of Apocrine Salivary Duct Carcinoma. Am J Surg Pathol 39: 744-752.
18. Williams L, Thompson LDR, Seethala RR, Weinreb I, Assaad AM, et al. (2015) Salivary Duct Carcinoma: The Predominance of Apocrine Morphology, Prevalence of Histologic Variants, and Androgen Receptor Expression. Am J Surg Pathol 39: 705-713.
19. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM (2012) Beyond PSA: The Next Generation of Prostate Cancer Biomarkers. Sci Transl Med 4: 127rv3-127rv3.
20. Robinson JLL and Carroll JS (2012) FoxA1 is a key mediator of hormonal response in breast and prostate cancer. Front Endocrinol (Lausanne) 3: 1-6.
21. Kusafuka K, Kawasaki T, Maeda M, Yamanegi K, Baba S, et al. (2016) Salivary duct carcinoma with rhabdoid features: a salivary counterpart against pleomorphic lobular carcinoma of the breast. Histopathology.

22. Nasser SM, Faquin WC, Dayal Y (2003) Expression of Androgen, Estrogen, and Progesterone Receptors in Salivary Gland Tumors - Frequent Expression of Androgen Receptor in a Subset of Malignant Salivary Gland Tumors. Am J Clin Pathol 119: 801-806.

23. Jin H-J, Zhao JC, Ogden I, Bergan RC, Yu J (2013) Androgen receptor-independent function of FoxA1 in prostate cancer metastasis. Cancer Res 73: 3725-3736.