Expression of Peroxisome Proliferator–Activated Receptor Gamma in Salivary Duct Carcinoma: Immunohistochemical Analysis of 15 Cases

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Salivary duct carcinoma is a rare but highly aggressive tumor of the salivary glands that has poor prognosis. There is no effective cure for this tumor. Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear receptor family with diverse biological functions that include mediation of adipocyte differentiation, regulation of the monocyte–macrophage anti-inflammatory activity, and inhibition of tumor cell proliferation. Natural (prostaglandin J_2, PG-J_2) and synthetic (thiazolinediones) PPARγ ligands with anti-proliferative agonist activity have been identified. The expression of PPARγ has been demonstrated in human colorectal, pancreas, breast, and prostate cancers but has never been explored in salivary duct carcinoma. The aim of our study was to investigate the expression patterns of PPARγ in salivary duct carcinoma, a finding that may provide a mechanism for treating patients with this highly aggressive tumor. Archival formalin-fixed tissues from 15 salivary duct carcinoma cases were analyzed for PPARγ expression by an immunohistochemical staining method using a monoclonal antibody against the PPARγ. The tissue sections were subjected to antigen retrieval by a steam heat method. All the cases of salivary duct carcinoma originated from the parotid gland. Immunohistochemistry analyses showed positive expression of PPARγ in 12 (80%) cases, whereas 3 (20%) were negative. Of the positive cases, 9 (75%), 2 (17%) and 1 (8%) showed strong, moderate, and weak staining, respectively. All staining was cytoplasmic. Nuclear staining was not observed. We conclude that PPARγ is frequently (80%) expressed in salivary duct carcinoma, often at high levels, and is topographically located in the cytoplasm. The high-level expression of PPARγ may provide a potential molecular target for the treatment of salivary duct carcinoma using agonist ligands.

KEY WORDS: Expression, Immunohistochemistry, Peroxisome proliferator-activated receptor, Salivary duct carcinoma, Treatment.

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Salivary duct carcinoma, first described by Kleinsasser *et al.* (1) in 1968, is a rare tumor of the salivary glands that was officially recognized by the World Health Organization in 1991 as a distinct clinicopathologic entity (2). Clinically, salivary duct carcinoma is characterized by its aggressive biologic behavior, and microscopically, it is characterized by resemblance to infiltrating ductal carcinoma of the breast (3). Many patients (>50%) with salivary duct carcinoma have lymph node metastases or show tumor extension into adjacent soft tissues at the time of presentation. The current main modality of treatment, surgical excision, has met with limited success, primarily because many tumors are at an advanced stage with early metastasis at the time of presentation and furthermore because the frequency of recurrence is high. An estimated 66% of patients with salivary duct carcinoma are dead within 4 years of diagnosis despite surgical resection (3). The response rate to radiation treatment is very poor, and currently there is no effective chemotherapy for this tumor. It is therefore imperative for investigators to explore alternative therapy for salivary duct carcinoma.

Recent studies have shown high levels of androgen receptor expression in salivary duct carcinoma, raising the possibility of using hormonal treatment

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in the management of these patients (4, 5). Patients with prostate carcinoma, which shows high frequency of androgen receptor expression, have been successfully managed by anti-androgen hormonal treatment (6). However, clinical studies have yet to be carried out in salivary duct carcinoma to evaluate the efficacy of such treatment. Prompted by the finding of androgen receptor expression in salivary duct carcinoma, we sought to further explore the expression patterns, if any, of another nuclear hormone receptor, peroxisome proliferator–activated receptor γ (PPARγ), in salivary duct carcinoma.

PPARγ is a member of the nuclear hormone receptor superfamily, which consists of a group of ligand-activated transcription factors that possess diverse biological functions (7, 8). The molecular structure of PPARγ shows a central DNA-binding domain, an amino-terminal activation domain (AF-1), a carboxyl-terminal ligand-binding domain (LBD), and a ligand-dependent activation domain (AF-2) (9). PPARγ forms a heterodimeric DNA-binding complex with retinoid X receptor, and both (PPARγ and retinoid X receptor) can be independently or coactivated by specific natural or synthetic ligands, leading to regulation of specific transcription genes (9–12). The natural ligands for PPARγ that have been identified include the potent eicosanoid 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (15d-PG J_2), and linoleic acid, whereas synthetic ones include thiazolidinediones such as troglitazone, rosiglitazone, and pioglitazone. The thiazolidinedione ligands are currently used as oral insulin sensitizers and anti-hyperglycemic drugs in patients with non–insulin-dependent diabetes mellitus (9, 13–15).

PPARγ is expressed in several tissues, including adipose tissue, where the gamma 2 isoform is preferentially expressed. In addition to the antidiabetic effect, ligand-activated PPARγ promotes adipogenesis and also regulates the monocyte–macrophage anti-inflammatory activity. Recent studies have also shown that PPARγ inhibits tumor cell proliferation and induces their terminal differentiation (16, 17). Expression of PPARγ has been demonstrated in several cancers, including colorectal, pancreas, breast, and prostate carcinoma (16–18). However, to our knowledge, no studies have been carried out to evaluate PPARγ expression in salivary duct carcinoma.

In this study, we investigated and demonstrated by immunohistochemical staining the expression of PPARγ in salivary duct carcinoma. The expression of PPARγ in salivary duct carcinoma may provide a therapeutic target, in a fashion similar to the treatment of hormone receptor–expressing tumors such as prostate carcinoma (anti-androgen) and breast carcinoma (anti-estrogen, tamoxifen).

MATERIALS AND METHODS

The surgical pathology files of the University of Arkansas for Medical Sciences and the University of Pittsburgh Medical Center were searched for cases of salivary duct carcinoma. Fifteen cases with available microscopic slides and suitable paraffin-embedded tissue blocks were identified for the period 1990 and 2000. Tissue from the resection tumor specimens, including portions of normal salivary gland, were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Fourteen patients were treated with parotidectomy with or without a neck dissection and/or radiation therapy. Only one patient was treated with radiation therapy alone. The clinical data and the histologic sections were reviewed to confirm the diagnosis.

Five-micrometer sections of formalin-fixed paraffin-embedded tissue were cut and prepared for IHC staining. The sections were incubated overnight at 4° C with a monoclonal antibody against human PPARγ (E-8; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:50 dilution. A case of prostate carcinoma with known expression of PPARγ was used as positive control, whereas negative controls were generated by replacement of the primary antibody with a matched primary antibody of unrelated specificity. Before incubation with the primary antibody, all sections were subjected to antigen retrieval by the heat steam method and were endogenous peroxidase quenched with blocking solution (Zymed Laboratories, San Francisco, CA). The standard avidin-biotin-peroxidase technique was used using the Zymed Kit Detection system (Zymed Laboratories). The immunostaining was assessed semiquantitatively by two pathologists (PM and CYF), and the results were scored as follows: no staining being *negative* (−); ≤20% positive cells being *weakly negative* (+); 30 to 40% positive cells being *mildly positive* (++); 50 to 70% positive cells being *moderately positive* (+++); and >70% being *intensely positive* (++++). For the purposes of this study, the expression of PPARγ is considered strong if the staining is scored as +++ or ++++; ++ and + represent moderate and weak expression, respectively; and ± or − indicate negative expression.

RESULTS

The clinical data and immunohistochemical staining results are summarized in Table 1. The tumors studied were from 10 men and 5 women, with an age range of 36 to 86 years (mean = 67.4 y). All of the 12 tumors arose from the parotid gland, and the majority of patients presented with Stage IV

TABLE 1. Clinical Data, PPARγ Immunostaining Results, Treatment, and Patient Follow-Up in 15 Salivary Duct Carcinomas Cases

Case #	Age/Sex	Tumor Location	TNM/Stage	IHC	Staining Extent	Parotidectomy/ Neck Dissection	Radiation Therapy	Recurrence/ Metastasis	Follow-Up
1	57/M	Parotid	T2N1/IV	+++	Diffuse	Yes/Yes	Yes	No	Alive/12 m
2	61/M	Parotid	T2N2/IV	++++	Diffuse	Yes/Yes	Yes	Local	Lost
3	66/F	Parotid	T2N2/IV	+++	Diffuse	Yes/Yes	Yes	No	Alive/13 m
4	58/M	Parotid	T3N0/II	+/−	Focal	No/No	Yes	Local	Dead/12 m
5	68/M	Parotid	T3N2/IV	+	Focal	Yes/Yes	Yes	Unknown	Lost
6	84/M	Parotid	T2N0/IV	++	Diffuse	Yes/Yes	Yes	Yes	Alive/30 m
7	67/F	Parotid	T1N0/I	++	Diffuse	Yes/Yes	Yes	No	Alive/34 m
8	37/F	Parotid	T1N0/I	+/−	Focal	Yes/Yes	Yes	No	Alive/44 m
9	77/M	Parotid	T2N0/I	+++	Diffuse	Yes/No	Yes	No	Alive/24 m
10	36/M	Parotid	T4N0/IV	+++	Diffuse	Yes/No	Yes	Local	Dead/10 m
11	78/M	Parotid	T4N2/IV	+++	Diffuse	Yes/Yes	Yes	Distant	Dead/6 m
12	86/F	Parotid	T3N2/IV	+++	Diffuse	Yes/Yes	Yes	Local	Dead/15 m
13	49/F	Parotid	T3N2/IV	+++	Diffuse	Yes/Yes	Yes	No	Alive/29 m
14	60/M	Parotid	TXN2/IV	+++	Diffuse	Yes/Yes	Yes	Distant	Dead/13 m
15	66/F	Parotid	T4N0/IV	−	N/a	Yes/Yes	Yes	Local	Lost

IHC, immunohistochemistry; TNM; tumor node metastases; N/a, not applicable.

disease. The duration of symptoms ranged from 2 to 9 months, and all patients presented with a neck mass around the parotid area. The masses were painless in all but three patients, in whom the pain was associated with recent enlargement. Other symptoms seen at presentation included VIIth nerve palsy (2 patients), vocal cord paralysis (1 patient), and cervical lymph node enlargement (7 patients). Radical parotidectomy was performed on 14 patients, but all patients received radiation treatment. At the time of radical resection of the tumors, 10 patients were found to have lymph node metastases and/or adjacent soft tissue involvement. Subsequent follow-up of the 15 patients for ≤44 months (mean = 20.2 mo) has shown that 5 died of their disease within 6 to 13 months, whereas 7 are alive with no evidence of disease and 3 patients have been lost to follow-up.

Pathologic Findings

On gross inspection of the resected specimens, the majority (12 of 15, 80%) of the tumors exhibited poor circumscription and showed extension into adjacent soft tissue. Cystic degeneration, necrotic foci, and hemorrhage were also seen in many tumors.

Microscopically, the tumors displayed the typical features, which have been described in salivary duct carcinoma (Fig. 1A). The tumor growth pattern was characterized by infiltrating ductal and intraductal components, with the latter demonstrating a variety of patterns including solid, papillary, cystic, and cribriform (Fig. 1C). The infiltrating ductal component showed small nests, cords, and single tumor cells embedded in dense desmoplastic collagenous stroma. Angiolymphatic and perineural invasion by tumor was present in many cases. In addition, foci of calcification were also noted. The individual tumor cells were predominantly characterized by nuclear enlargement, with hyperchromasia, prominent nucleoli, variably increased nuclear to cytoplasmic ratios, abundant amphophilic or eosinophilic cytoplasm, and frequent mitoses.

Immunohistochemical Detection of PPARγ

The immunohistochemical staining of salivary duct carcinoma showed positive diffuse staining for PPARγ in the majority of cases (12 of 15, 80%), with 75% (9 of 12) of the cases showing strong staining (Fig. 1B). Two (17%) and 1 (8%) of the cases demonstrated moderate and weak staining for PPARγ. Only 3 (20%) of total cases studied were negative for PPARγ expression (Fig. 1C). Of the positive cases, the staining pattern was uniform throughout the tissue section, with almost all neoplastic cells showing positive staining in the cytoplasm of most cases (with minimal or no nuclear staining). In two cases, the staining for PPARγ displayed a granular cytoplasmic pattern (Fig. 1C). Cytoplasmic staining of the PPARs has been described before (19). In addition, positive cytoplasmic staining in the striated and intercalated ducts of the normal salivary glands was also noted, whereas the acinar and stromal cells were negative, thus providing good internal control for the immunohistochemical staining (Fig. 2).

Statistical Analysis

Statistical analysis, in our small series of salivary duct carcinoma, showed no correlation between the level of PPARγ expression and tumor stage (TNM and clinical), recurrence, or patient survival.

DISCUSSION

The current main treatment modality for salivary duct carcinoma is complete surgical excision fol-

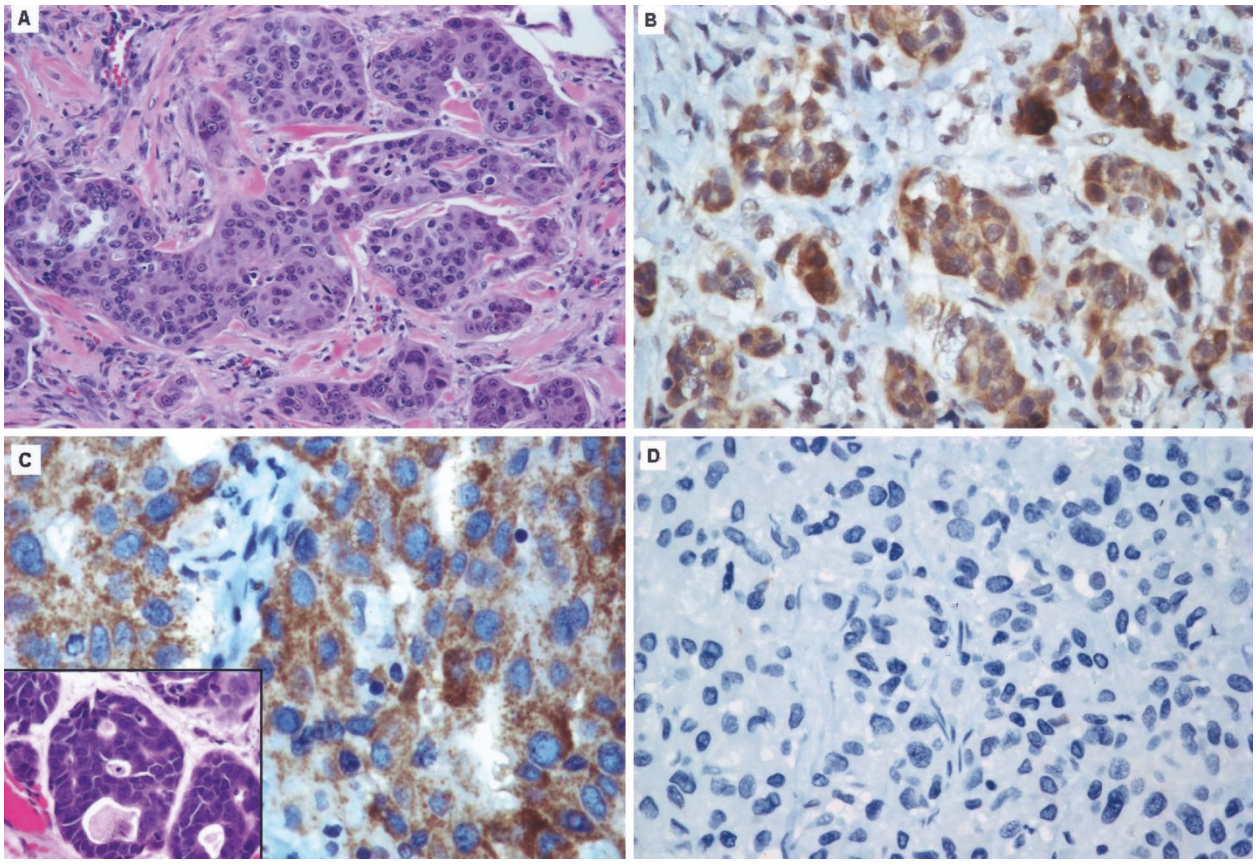

FIGURE 1. **A**, histologic sections of invasive salivary duct carcinoma showing tumor cells with atypical nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm. **B**, immunoperoxidase staining for PPARγ in salivary duct carcinoma showing strong positive diffuse cytosolic expression. **C**, tumor (glandular and cribriform pattern) displaying granular cytoplasmic staining (***inset***, hematoxylin-eosin section of tumor with cribriform pattern). **D**, one of three cases negative for PPARγ expression.

lowed by radiation therapy (4). However, salivary duct carcinoma frequently presents at an advanced stage (Stage IV for most patients in our study), therefore leading to treatment failure in most cases. Approximately 60% of the patients with salivary duct carcinoma die within 4 years of initial diagnosis (3, 4). Currently, there is no effective chemotherapy for this malignancy, thus making the management of patients with distant metastases difficult.

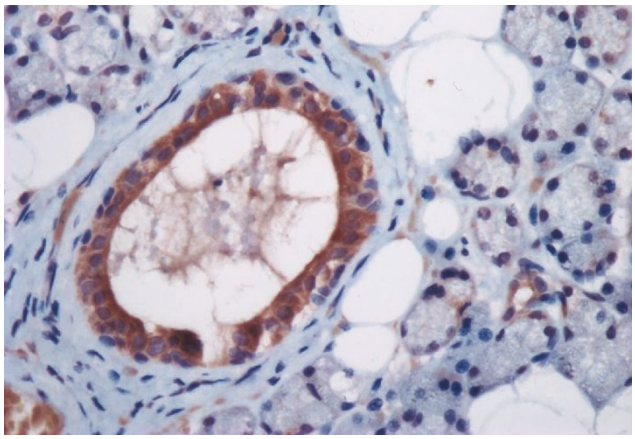

FIGURE 2. Normal salivary gland tissue; expression of PPARγ is present only in the ductal epithelium. Acinar cells and stromal tissue do not express PPARγ.

We observed in our study high levels of PPARγ expression in many salivary duct carcinoma, 12 of 15 (80%), and that expression was predominantly limited to the cytoplasm. Our interest in investigating the expression of PPARγ in salivary duct carcinoma arose from the observation that salivary duct carcinoma express androgen receptor, which is also a nuclear hormone receptor (4, 5). In a previous study, androgen receptor expression was seen in ≤92% of salivary duct carcinoma, leading to speculation that hormonal treatment may have a role in the management of this tumor (4). Therapy targeted at hormone receptors has been successfully used in the treatment of prostate carcinoma (androgen ablation) and breast carcinoma (tamoxifen), both nuclear hormone receptor–expressing tumors. Salivary duct carcinoma shows some immunophenotypic similarity to prostate carcinoma, like prostate carcinoma and in addition to androgen receptor expression; it also expresses prostate-specific antigen and prostate acid phosphatase (20). Salivary duct carcinoma bears microscopic resemblance to breast carcinoma, which itself shows spo-

radic androgen receptor expression; and it is therefore not surprising that investigators have looked for estrogen and progesterone receptor expression in salivary duct carcinoma. However, estrogen and progesterone receptor expression in salivary duct carcinoma appears to be infrequent (5, 6, 21, 22). Another notable finding was the demonstration of PPARγ expression in both prostate carcinoma and ductal carcinoma of the breast (23–25). The expression of PPARγ in salivary duct carcinoma suggests that it may be possible to specifically target this receptor in the treatment of patients with this tumor.

The antiproliferative effect of PPARγ is mediated through the inhibition of the cyclin D1 gene. Wang *et al.* (9) demonstrated that when PPARγ is activated by 15d-PG J_2, PGD_2 or by the synthetic ligand troglitazone, the result was inhibition of *cyclin D1*, blocking entry of the cell cycle into the S phase. This effect was not observed in cells that were deficient of PPARγ (26). The antiproliferative effects of PPARγ ligands have been observed in breast, colon, and recently, in pancreatic carcinoma (18, 27). In addition, PPARγ ligands also induce terminal differentiation in fibroblasts, human breast cancer, and liposarcoma cells. In the latter case, PPARγ ligands have been shown to arrest proliferation of liposarcoma cells and promote their maturation into adipocytes (16, 28, 29).

A contradictory biologic effect was the observation in murine studies that troglitazone-activated PPARγ promoted tumorigenesis in the colon (27, 30–32). It is speculated that the apparent tumorigenic activity of PPARγ in the colon in these animals may be related to its anti-inflammatory effects, whereby a diminished inflammatory function may lead to a decrease in tumor surveillance (9, 31, 33).

In our study, PPARγ expression was predominantly cytoplasmic. Shibuya *et al.* (34) demonstrated that nitration of PPARγ in macrophage cell lines (RAW 264), resulted in inhibition of the ligand-dependent translocation of PPARγ into the nucleus. Normally PPARγ-ligand binding occurs in the cytosol, followed by translocation into the nucleus. Whether PPARγ in salivary duct carcinoma is nitrated or not or the translocation into the nucleus is precluded by a different mechanism remains unknown and requires further investigation.

Although PPARγ expression did not correlate with tumor stage, recurrence, or survival, its significance is that it provides a potential target site for therapeutic manipulation.

In summary, we have demonstrated a high level of expression of PPARγ in most salivary duct carcinoma. The diverse biologic functions of PPARγ and the mechanisms of specific ligand activation are complex and are yet to be fully understood. However, the expression of PPARγ in salivary duct carcinoma and other tumors offers a potential therapeutic target for developing drugs that specifically bind and activate this receptor to alter tumor growth.

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