Anticancer Activity of Docetaxel in Murine Salivary Gland Carcinoma

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ABSTRACT

Purpose: The purpose of this study was to evaluate the biological mechanisms of docetaxel (TXT) on salivary gland carcinoma.

Experimental Design: The effects of TXT on a spontaneous murine salivary carcinoma were determined. Proliferation, cell cycle regulation, connexin43 expression, gap-junctional intercellular communication, apoptosis, and Fas receptor (FasR) expression were measured.

Results: We characterized a spontaneous mouse salivary gland carcinoma (SGC1). SGC1 is a poorly differentiated carcinoma that originated from the parotid gland of a BALB/c mouse. SGC1 cells were cultured and found to be immortal past 30 passages. Initially, cells formed tumor nodules in severe combined immunodeficient (SCID) mice. Afterward, SGC1 cells that were subcultured from SCID tumors readily formed colonies in soft agar and were highly tumorigenic in SCID mice and immune-competent BALB/c hosts. Dose response for TXT with respect to growth suppression, G2-M cell cycle arrest, and apoptosis was found. Induction of apoptosis by TXT coincided with an increase in cell surface FasR expression. Up-regulation of FasR with lower doses of TXT rendered cells susceptible to FasR agonist antibody-mediated apoptosis. In the absence of TXT, anti-FasR antibodies were completely without effect, suggesting that TXT is critical for priming apoptosis mediated through the Fas pathway. In addition, gap-junctional intercellular communication was augmented by TXT in SGC1 cells concomitant with increased connexin43 expression and membrane localization.

Conclusions: We have identified several novel targets of TXT that contribute to its antitumor activity in poorly differentiated salivary gland carcinoma. These results suggest that TXT may be appropriate for additional in vivo studies and clinical trials in patients with salivary cancers.

INTRODUCTION

The standard treatment for major salivary gland cancers is surgical resection; however, a substantial risk of local-regional recurrence (16–27%) and distant metastasis (13–26%) exists (1–3). Adjuvant radiation has been used in select cases in an attempt to reduce the local-regional failure rate (4). However, the high rate of distant metastasis has not been addressed by these local modalities of therapy. Although chemotherapy has been used for unresectable disease and to palliate local-regional recurrence, the role of adjuvant chemotherapy has not been clearly defined in preventing distant metastasis. In our previous series of patients with salivary gland cancer, we found that receipt of both adjuvant radiation and chemotherapy was an independent predictor of an improved disease-free survival (P = 0.05; Ref. 5). Furthermore, distant metastasis (28%) was a more common site of progression as opposed to local-regional recurrence (19%; Ref. 5). High-risk histopathological features were predictive of disease failure (1–3, 5).

The use of chemotherapy for recurrent and/or unresectable salivary gland carcinoma has been tested. Cisplatin, doxorubicin, and 5-fluouracil are active agents against malignant salivary gland tumors (6, 7). Combination chemotherapy including the aforementioned drugs with mitomycin C, cyclophosphamide, methotrexate, bleomycin, paclitaxel, vincristine, and vinorelbine has also demonstrated a tumor response (6–10). TXT is a newer taxane that has not been tested in salivary gland cancers. TXT has significant antitumor effects, and it is currently being tested in patients with head and neck squamous cell carcinoma (11–13). TXT is an antimicrotubulin agent that promotes tubulin assembly, inhibits depolymerization, acts as a mitotic spindle poison, and induces mitotic block in proliferating cells (14). TXT induces G2-M arrest and p53-independent apoptosis in various cancer cell lines. However, the antitumor effects of TXT in a salivary gland cancer model have not been evaluated. Therefore, we measured the effects of TXT on proliferation, cell cycle progression, Cx43 expression, GJIC, apo-

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3 The abbreviations used are: SCID, severe combined immunodeficient; PCNA, proliferating cell nuclear antigen; GJIC, gap-junctional intercellular communication; TXT, docetaxel; Cx43, connexin43; BrdUrd, bromodeoxyuridine; MCF, mean channel fluorescence; TBST, 10 mM Tris-HCl (pH 8.0), 50 mM NaCl, and 0.1% Tween 20; FasR, Fas receptor; CCD, charge-coupled device; FasL, Fas ligand.
ptosis, and FasR expression in a novel salivary gland cancer model.

MATERIALS AND METHODS

TEXT and Cell Lines. TEXT (Aventis Pharmaceuticals, (Vitry Sur Serine Cedex, France) was suspended in ethanol at 10 mg/ml and stored at −20°C. Dilutions were prepared in culture medium to make final concentrations of 5, 10, 20, 40, 60, 80, and 100 mg/ml. The final ethanol concentration was <0.05% by volume. The murine salivary gland carcinoma cell line SGC1 was derived from a spontaneous salivary tumor that arose in a BALB/c mouse that was bred in house at the Karmanos Cancer Institute Animal Facility. The SGC1 cell line was maintained in vitro in DMEM supplemented with 10% heat-inactivated Serum Supreme (BioWhitaker, St. Louis, MO), 0.5 mM sodium pyruvate, 2 mM L-glutamate, 0.1 mM MEM nonessential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin. Derivatives of the parental SGC1 tumor were subcultured from primary tumor tissue after two serial passages in SCID mice (SGC1-sc1 and SGC1-sc2) and immune-competent BALB/c (SGC1-ica1) mice. All in vitro data presented in this study used the SGC1 cell line derived from the original parental tumor between passages 10 and 20.

Immunohistochemistry. The primary tumor, normal margin tissue, and the unaffected normal parotid from the opposite side were resected, rinsed in sterile saline, fixed in 10% neutral buffered formalin, and embedded in paraffin using standard histochemical techniques. Tissue sections (3–4 μm) were stained with H&E for basic histo logical examination. Immunohistochemical detection of high molecular weight cytokeratins (clone 34BE12, Zymed Laboratories, Inc. second generation reagent) or PCNA (biotinylated PC10 staining kit; Zymed Laboratories, Inc., South San Francisco, CA) was done according to the recommended procedures, and samples were developed with 3,3′-diaminobenzidine substrate (Zymed Laboratories, Inc.) and counterstained with hematoxylin. Samples were evaluated with an Olympus BX-40 microscope equipped with a CCD Sony DXC-970 MD color video camera. Photomicrograph images were acquired with MCID5+ imaging software package.

Proliferation Assay. Proliferation rates were determined by measuring BrdUrd incorporation using the BrdUrd Proliferation Assay Kit (Oncogene Research Products, Boston, MA). Quadruplicate wells of SGC1 cells were plated in flat-bottomed 96-well plates at 10,000 cells/well in 100 μl of media until cells reached 60–70% of confluence. TEXT (10, 20, 40, 60, or 80 ng/ml) or solvent alone was added to fresh media (total volume 2 ml of media until cells reached 60–70% of confluence). Fresh medium was added that contained different concentrations of TXT or solvent alone. Cells were cultured in the presence of drug for an additional 48–72 h before harvest. Cells were washed with cold PBS and concentrated (1 × 10⁶ cells in 0.1 ml of PBS). An equal volume of ice-cold ethanol was added while vortexing the sample. The sample was washed with PBS and centrifuged for 30 min at 1500 rpm, resuspended in 0.3 ml of PBS containing 1 mg/ml DNase-free RNase I, and incubated at 37°C for 30 min before the addition of propidium iodide (20 μg/ml final concentration). Cell cycle distribution was measured with a FACsCaliber flow cytometer (Becton Dickinson, Mountain View, CA). At least 10,000 events per sample were acquired. The ModFit LT (Verity Software House, Inc.) cytological software program was used for data analysis.

Apoptosis. An annexin V-FITC apoptosis detection kit (BD PharMingen) was used for apoptosis detection. After TXT treatment, attached cells were treated briefly with trypsin, quenched with media, washed once with cold PBS, and then resuspended in chilled binding buffer and annexin V-FITC according to the kit instructions. After the 15-min incubation at room temperature, propidium iodide was added to label the nonviable cells. Samples were placed on ice and evaluated immediately by dual-color flow cytometry (FL1 versus FL2). A total of 10,000–20,000 events were collected per sample. Data were analyzed using WinMDI version 2.8 software.

Evaluation of Cell Surface FasR Expression by Flow Cytometry. FITC-conjugated hamster IgG anti-FasR (clone Jo2; PharMingen) was used to detect cell surface expression of the FasR in SGC1 cells after treatment with TXT. Samples were stained with FITC-conjugated anti-FasR antibody or isotype control and diluted 1:40 in staining buffer (PBS supplemented with 2% serum and 0.1% sodium azide) on ice for 45 min. Samples were analyzed by flow cytometry using a FACsCaliber flow cytometer.

To determine whether the FasR agonist antibody Jo2 could further enhance apoptosis through the FasR pathway, SGC1 cells were subcultured in 6-well plates as described above and treated for 48 h with TXT at 0, 10, or 20 ng/ml in duplicate wells. One set of wells was treated with an isotype control antibody, and the other set received 5 μg/ml anti-Fas agonist antibody Jo2 and incubated for an additional 20 h. Cells were harvested and processed for annexin V-FITC binding and apoptosis (above).

GJC. GJC was tested by using red and green fluorescent donor cell labeling and flow cytometric analysis of dye transfer to unlabeled recipient cells with a slight modification to a previously described protocol (14). Donor SGC1 cells that were grown for 48 h in the absence or presence of 20 ng/ml TXT were dual-labeled in solution A (PBS, 30 mM HEPES and 10 mM glucose) supplemented with 0.5 μM calcein acetoxyethyl ester (Molecules Probes, Eugene, OR; 1:2000 from a 1 mM stock in DMSO) and 9 mM diolkecarboxyamine dye (Molecules Probes; 10 mM stock in DMSO).

Briefly, medium was aspirated, replaced with 2 ml of dye-supplemented solution A, and incubated for 30–60 min at 37°C. Cells were rinsed in solution A, trypsinized, and monodispersed in complete media. Graded numbers of donor cells were added to freshly plated recipient cells that were also grown (in parallel) in the absence or presence of 20 ng/ml TXT.
Recipient cells were plated in 2 ml of complete media in 6-well plates with $1 \times 10^6$ cells/well just before the addition of donor cells. Donor cells were added to achieve donor:recipient ratios of 1:2, 1:5, 1:10, or 1:20 for 4–5 h at 37°C. Calcein transfer in live cells was periodically monitored by an Olympus IX-FLA inverted reflected light fluorescence microscope equipped with a dual filter cube for simultaneous two-color analysis and imaged using a SPOT CCD camera with imaging software V 3.0 (Diagnostic Instruments, Inc., Sterling Heights, MI). Photomicrographs were acquired using the $\times 40$ objective just before flow cytometric analysis. For quantitative analysis of dye transfer, cells were harvested and evaluated by flow cytometry. Histograms for quantitating the level of calcein transferred to the recipient cells (GJIC and coupling efficacy) are reported as the MCF.

Immunofluorescence of Cx43. SGC1 cells were plated on glass coverslips in 6-well plates in complete media. One day after plating when cells were approximately 60% confluent, media were replaced with fresh media containing various concentrations of TXT and incubated for an additional 48 h. After treatment, cells were rinsed once with PBS and then fixed with ice-cold methanol at $-20^\circ$C for 20 min. Coverslips were air dried, treated with PBS containing 0.125% Triton X-100, 8% serum, and 0.1% sodium azide for 20 min at room temperature, and washed three times (5 min each) with staining buffer. Primary monoclonal antibody to Cx43 raised against a peptide antigen between residues 252 and 270 (Transduction Laboratories, Lexington, KY) was diluted 1:25 (10 $\mu$g/ml) in staining buffer, applied directly to the coverslip, incubated at room temperature for 1 h, and labeled with secondary rhodamine (tetramethylrhodamine isothiocyanate)-conjugated goat antimouse IgG (1:100; Jackson ImmunoResearch, West Grove, PA) for 45 min at room temperature in the dark. Samples were visualized at $\times 100$ under oil with an Olympus BX-40 microscope equipped with a CCD3 Sony DXC-970 MD color video camera. Fluorescence photomicrographs were imaged using MCID5+ software.

Immunoprecipitation and Western Blot Analysis. Lysates were prepared from monolayer cultures after washing them twice with ice-cold PBS and harvested by scraping, pelleting, and resuspension in ice-cold lysis buffer [50 mM HEPES (pH 8.0), 10% glycerol, and 1% Triton X-100] supplemented with protease (Oncogene Sciences) and phosphatase inhibitor mixtures (Sigma Chemical Co.). Cell lysates were incubated on ice for 60 min with occasional mixing and clarified by centrifugation at 16,000 $\times$ g for 20 min at 4°C. Cx43 protein was immunoprecipitated with 2 $\mu$g of anti-Cx43 mouse monoclonal antibody (CX-1B1; Zymed Laboratories) for 2–4 h before the addition of protein A/G Plus-agarose (Santa Cruz Biotechnology). Samples were rotated at 4°C for 16–18 h, washed twice with lysis buffer, eluted in 1X sample buffer, boiled for 3 min before fractionation in 12% SDS-PAGE, and transferred to Immobilon-P (Millipore, Bedford, MA) polyvinylidene difluoride membranes. Membranes were blocked overnight at 4°C in TBST buffer with 1% BSA. Cx43 protein was detected by immunoblotting with monoclonal antibody CX-1B1 diluted 1:1000 (0.5 $\mu$g/ml) in TBST + 1% BSA, followed by goat antimouse horseradish peroxidase (Jackson Immunoresearch) diluted 1:10,000 in TBST + 1% BSA. Blots were developed with enhanced SuperSignal West Pico Chemiluminescent Substrate (Pierce) and Kodak-MR film.

Tumor Growth in SCID and Immune-competent BALB/c Mice. Female CB17 SCID mice (6 weeks of age) were obtained from Harlan (Frederick, MD) and challenged s.c. in the inguinal area with $5 \times 10^6$ SGC1 cells suspended in 0.1 ml of Matrigel (Becton Dickinson, Bedford, MA). All in vivo tumor growth studies were done in strict compliance with Division of Laboratory Animal Resources and institutional guidelines. Tumor growth was monitored by weekly palpation. Tumors that developed in SCID mice were resected aseptically, recultured in vitro, and reimplanted into SCID mice and immune-competent syngeneic BALB/c mice to test for tumorigenicity. SGC1 cells from this primary passage in SCID mice were also grown in 0.35% soft agar to evaluate anchorage-independent growth potential.

RESULTS

Spontaneous BALB/c Salivary Gland Carcinoma. A 56-week-old BALB/c female presented with a rapidly growing right upper neck mass. At the time of sacrifice, the lesion was noted to be growing out from the parotid gland. The tumor was removed along with the grossly normal-appearing parotid and submandibular glands. A portion of the tumor was explanted and adapted for growth in vitro, and the remaining specimen was prepared for histological analyses. Histological analysis revealed a poorly differentiated carcinoma that originated from a normal parotid gland (Fig. 1A). The neoplasm consisted of solid nests of polygonal to spindle-shaped tumor cells undergoing frequent central necrosis and separated by a delicate stromal network. Tumor cells had sharply defined cell borders, a moderate amount of eosinophilic cytoplasm, a high nucleocytoplasm ratio, and hyperchromatic and pleomorphic round to elongated nuclei (Fig. 1B), with frequent nucleoli and abundant mitotic figures (Fig. 1B).

Expression of Keratin, S100, PCNA, and Common and Muscle-specific Actin. The epithelial nature of the neoplasm was confirmed by expression of high molecular weight keratins using 34BE12 (Fig. 1C). Little reactivity is observed in the normal ductal and acinar cells (data not shown). However, the simple epithelial cells in the normal tissue were intensely reactive with the AE3/AE1 pan cytokeratin antibody mixture (data not shown). Intense reactivity with LL002 anti-keratin 14 antibody and moderate reactivity with the pan cytokeratin AE3/AE1 antibody mixture were also observed in the tumor tissue (data not shown). SGC1 cells were nonreactive with S100 and common and muscle-specific actin and weakly reactive with vimentin (data not shown). The PCNA index in the carcinomaous tissue was approximately >50%, whereas in the adjacent normal parotid acinar and ductal cells, it was <10% (data not shown). PCNA index in high-grade malignant parotid tumors has been correlated with higher local recurrence and mortality (15). The combined immunohistochemical and morphological features of the neoplasm were compatible with the diagnosis of high-grade carcinoma with sarcomatoid features (sarcomatoid carcinoma). Neoplasms with similar morphology occur in human salivary glands, including the parotid, where they can either present ex novo or as a malignant recurrence of benign mixed...
tumors (16). Although this specific entity is rare in humans, it is representative of a group of undifferentiated high-grade carcinomas that have a high risk for local regional recurrence and distant metastases (5). With its aggressive features, SGC1 is an appropriate model to test drug efficacy because this tumor would be managed by surgery and chemotherapy, similar to the more common high-grade mucoepidermoid and adenoid cystic carcinomas.

**In Vitro and in Vivo Growth of SGC1, SGC1-sc1, SGC1-sc2, and SGC1-ica1.** Explanted tumor cells grew readily in DMEM and 10% serum, and the cells were stable in culture for >30 passages. SGC1 cells are not contact inhibited and often form two to three dense layers of cells that can be sloughed sequentially with increasing concentrations of trypsin. The saturation density of one such culture is $1 \times 10^6$ cells/cm². SGC1 cells express high levels of epidermal growth factor receptor, MHC class I (H2-K¹), and Fas, whereas MHC class II I-A¹ was not detected (data not shown). We tested tumorigenicity in vivo by injecting SGC1 cells (cultured for 10 passages) into SCID mice. After a 2–3-month latency period, three of four animals developed tumor nodules at the site of injection. Morphologically and histologically, tumor cells and tissue recovered from the SCID mice were indistinguishable from the parental SGC1 tumor and cell line. After a primary expansion in vitro, the SCID-derived SGC1 cell line (SGC1-sc1) was reinjected into SCID mice. All (four of four) injection sites had rapidly (within 4–5 weeks) growing tumor nodules reaching an average cross-sectional area of 100 mm². This derived cell line was designated SGC1-sc2. Approximately 50% of SGC1-sc2 cells demonstrated anchorage-independent growth and formed foci (20–2000 cells/colony) in 0.35% top agar within 7–10 days. When SGC1-sc2 cells were injected into immune-competent BALB/c (H2-K¹⁺) mice, tumor nodules were palpable at four of four (100%) injection sites within 2 weeks. These nodules were indolent for 2 weeks and then grew rapidly to >80 mm² by 6 weeks after injection.

**Inhibition of Proliferation and G₂-M Cell Cycle Arrest of SGC1 by TXT.** All in vitro analyses were performed on the primary cell line, SGC1. After exposure of SGC1 cells to TXT, proliferation was suppressed significantly ($P < 0.001$) at concentrations higher than 20 ng/ml (Fig. 2). This is in line with clinically relevant concentrations (17) and clearance kinetics observed in patients (18). Growth suppression of ≥75% was noted at concentrations of ≥40 ng/ml. SGC1 cells were arrested in G₂-M by TXT in a dose-dependent manner (Fig. 3). Therefore, growth inhibition of the SGC1 cell line is predominately because of G₂-M arrest as described in other tumor types. By immunofluorescence and Western blot analyses, SGC1 cells expressed normal levels of nuclear retinoblastoma, cyclin D1, and cyclin-dependent kinase 4. Although not detected by Western blot, the p53 status appears normal by sequence analysis from genomic DNA of exons 5–8.

**Induction of Apoptosis (Annexin Binding) and Fas Expression in SGC1 by TXT.** A dose-dependent increase in apoptosis (percentage of annexin binding) induced by TXT in SGC1 cells was observed (Fig. 4A). An increase in the level of FasR expression on the cell surface also paralleled apoptosis (Fig. 4B). FasL was expressed at low to undetectable levels in SGC1 cells and was not increased by TXT (data not shown). To determine the functional consequence of Fas up-regulation by TXT, apoptosis was measured in SGC1 cells after anti-Fas agonist antibody was added to TXT-pretreated cells. At low concentrations (10 and 20 ng/ml) of TXT, the addition of the agonist Fas antibody more than doubled the level of apoptosis as compared with treatment with TXT alone (Fig. 5). Therefore, TXT exposure sensitizes SGC1...
cells to anti-Fas agonist antibody-mediated apoptosis and suggests that this is due, in part, to an up-regulation in cell surface Fas expression. Interestingly, the anti-Fas agonist antibody alone had only a marginal effect on apoptosis. We cannot exclude the possibility that enhancement of this pathway of apoptosis may also be related to (secondary to) the effect of TXT on cell cycle and stress response pathways, which make the cell more vulnerable to Fas-mediated apoptosis or alterations in the transport of Fas due to microtubule stabilization. Although we did not examine the Bcl-2 apoptosis pathway, we suspect that the overall incidence of TXT-induced apoptosis in SGC1 cells probably has significant bcl-2 and bax components, as it does in other tumor types (19). The more novel up-regulation of FasR by TXT and sensitization to apoptosis through this pathway may have additional implications for therapy of tumors with FasR expression.

**GJIC and Expression of Cx43.** We initially observed that treatment of subconfluent SGC1 cultures with low levels of TXT restored cell contact inhibition and reduced the saturation density of the culture. Therefore, we tested whether the establishment of stable gap junctions was responsible for this phenomenon.

Therefore, we examined the effects of microtubule stabilization by TXT on GJIC along with Cx43 expression and localization. GJIC was measured by green fluorescent dye (calcein AM) transfer between donor (red and green) and recipient cells (green only). After TXT exposure (20 ng/ml for 48 h), almost all recipient cells surrounding donor cells were intensely positive with green fluorescence (Fig. 6A, bottom panel). SGC1 control donor and recipient cells demonstrated weak dye transfer (Fig. 6A, top panel). When quantitated by flow cytometry, the MCF peak shifted from 37 (control) to 68 (TXT-treated cells; Fig. 6B), reflecting a 2-fold increase in the amount of calcein transferred from donors to recipients. Because functional coupling requires connexin expression

**Fig. 2** Inhibition of proliferation is measured by BrdUrd uptake of SGC1 cells after exposure to various concentrations of TXT for 24, 48, 72, and 96 h. Data are presented as percentage inhibition relative to untreated or solvent-treated control wells for each day (the ratio of the average absorbance of the control to the average absorbance of the treatment group; n = 4 wells/data point). •, 10 ng/ml; ■, 20 ng/ml; —, 40 ng/ml; ▲, 60 ng/ml; +, 80 ng/ml; ○, 100 ng/ml.

**Fig. 3** G2-M arrest induced by TXT. Cell cycle arrest of SGC1 cells after 48 h of exposure to increasing doses of TXT. The percentage of cells in G2-M as calculated by ModFit cell cycle analysis software is represented in bar graph form.

**Fig. 4** Apoptosis and Fas expression induced by TXT. A, the percentage of annexin-FITC-positive cells and (B) the MCF value for anti-FasR-FITC antibody binding were measured by flow cytometry 48 h after exposure to increasing concentrations of TXT in SGC1 cells.

**Fig. 5** Apoptosis induced by TXT and anti-Fas agonist. The percentage of annexin V-FITC binding was measured in SGC1 cells by flow cytometry after a 48-h exposure to TXT (10 and 20 ng/ml) and an additional 20 h with Jo2 (5 µg/ml) anti-Fas (□) or isotype control antibody (■).
and membrane localization, we analyzed Cx43 protein expression in situ after TXT exposure (Fig. 6C). The untreated control cells demonstrate low levels of Cx43 immunoreactive foci arrayed randomly in cellular processes and at cell-cell junctions (Fig. 6C, top panel). After treatment with TXT, Cx43 immunoreactivity increased and localized to the plasma membrane at cell-cell junctions (Fig. 6C, bottom panel). Junctional plaques appeared to be enriched, more abundant, and more uniformly dispersed along the entire edge of cell-cell borders (Fig. 6C). Furthermore, TXT treatment increased total cellular Cx43 protein levels as demonstrated by immunoprecipitation and Western blot analysis of Triton X-100-soluble (Fig. 6D) and whole cell lysates (data not shown). In untreated SGC1 cells, total cellular Cx43 is low, even after enrichment by immunoprecipitation (Fig. 6D, lane 1). SGC1 cells treated with TXT (20 ng/ml, 48 h) exhibit a marked enhancement in Cx43 expression (lane 2). Thus, we found that TXT induced intercellular coupling by the connexin pathway via an increase in Cx43 protein levels, plasma membrane localization, and the formation of junctional plaques and functional channels capable of dye transfer between cells, a novel finding that has not been demonstrated previously.

**DISCUSSION**

This is the first report of a cell line (SGC1) isolated from a poorly differentiated salivary gland carcinoma (sarcomoid
carcinoma) occurring spontaneously in a BALB/c mouse that was capable of both in vitro and in vivo growth. Salivary gland cancers occur spontaneously at a low frequency (<1%) in BALB strains of mice and are primarily myoepitheliomas (20). We proceeded to characterize the SGC1 cell line to understand the mechanisms contributing to its tumorigenic phenotype so that putative treatment modalities could be considered. Because SGC1 had a rapid growth rate in culture and easily established tumor nodules in SCID and immune-competent BALB/c mice, we wanted to evaluate an agent that would inhibit growth in this tumorigenic cell line. We examined the effects of a newer chemotherapy agent, TXT, not yet tested in salivary cancer. We have demonstrated that the antiproliferative effect of TXT is secondary to G2-M arrest and induction of apoptosis in SGC1 cells, similar to other cancer types.

In murine salivary carcinoma cells, we demonstrated that TXT induces apoptosis through the Fas pathway. TXT increases Fas expression, and the addition of an anti-Fas agonist antibody augments TXT-induced apoptosis. Taxanes promote apoptosis by altering expression of the Bcl-2 family and signal transduction pathways of mitogen-activated protein kinase (c-Jun NH2-terminal kinase/stress-activated protein kinase) and by increasing secretion of cytotoxic cytokines (21). Sensitization to Fas-mediated cytotoxicity has been observed with several cytotoxic agents, including cisplatin and 5-fluorouracil in human bladder cancer cells (22, 23), camptothecin in prostate cancer cells (24), and all-trans retinoic acid and cisplatin in medulloblastoma cells (25). In some instances, these effects were mediated by modulation of Fas expression; however, most studies state that drug-induced apoptosis is not mediated by the Fas/FasL signaling pathway (26, 27). Paclitaxel was shown to augment FasL-induced apoptosis in glioma cells; however, this effect was mediated by bcl-2 regulation of the Fas pathway (28). TXT has previously been found to induce apoptosis by bcl-2 phosphorylation (19, 29), which leads to the inactivation of its antiapoptotic signal and increased apoptosis.

TXT exposure increases Cx43 expression, the formation of gap-junctional plaques, and functional GJIC (i.e., intercellular communication dye transfer) in SGC1 cells. This enhancement of GJIC and connexin expression may thus constitute a novel therapeutic target of TXT. Connexins are proteins that form gap junction channels. GJIC plays a crucial role in cellular homeostasis, and alterations in GJIC are found during tumor promotion and progression (30). GJICs link single cells within a population to facilitate the passage of small regulatory molecules (M, 1200) and cytoplasmic ions to maintain metabolic homeostasis. Loss of GJIC and/or down-regulation and inactivation of connexins have been observed in a variety of cancers (30). When restoration of GJIC was achieved by either pharmacological agents (31) or connexin gene transfer (32), tumor cell killing by conversion of ganciclovir to its cytotoxic form by thymidine kinase was enhanced. In glioblastoma cells, Cx43 reexpression enhanced paclitaxel-induced apoptosis (33). Furthermore, connexin gene transduction results in reversion of neoplastic phenotype and inhibition of tumorigenic potential in experimental models (34). Our results suggest that TXT can be tested on salivary cancers. After surgery and radiation therapy for advanced and high-risk salivary gland cancers, patients have high rates of distant failures (13–28%) and local-regional recurrence (16–27%); Refs. 1–3). Only systemic chemotherapy will address distant sites of failure. High-risk pathological features have been associated with poor prognoses and a high rate of local-regional and distant failures (5). Although the role of chemotherapy in recurrence of salivary cancers has been established (6–10), the use of adjuvant chemotherapy for high-risk patients is being tested. In our previous series, we observed that adjuvant chemotherapy and radiation therapy may improve disease-free survival (P = 0.05; Ref. 5).

In conclusion, limited data exist concerning both the in vitro and in vivo effects of TXT in salivary gland cancers. In addition to the known antitumor mechanisms of TXT, such as induction of growth arrest, G2-M arrest, and apoptosis, we have shown that TXT increases GJIC by increasing the expression of Cx43 in murine salivary gland carcinoma cells. Furthermore, we have shown that TXT promotes apoptosis in part through the Fas pathway in murine SGC1 cells. These results suggest that TXT may be appropriate for additional in vitro and clinical trials in patients with salivary cancers. It is tempting to speculate that the effects of TXT as an antimicrotubulin agent on GJIC, FasR expression, cell cycle arrest, and apoptosis are all interrelated. Understanding the relationships among these pathways could lead to improved strategies that exploit these mechanisms for therapeutic intervention.

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