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A novel combination of withaferin A and sorafenib shows synergistic efficacy against both papillary and anaplastic thyroid cancers

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Abstract

BACKGROUND: Sorafenib (SO), a multikinase-targeted inhibitor in clinical trials for papillary and anaplastic cancers, shows limited efficacy with moderate toxicity. Withaferin A (WA), a natural withanolide, shows potent preclinical anticancer activity in thyroid cancers through multiple cytotoxic mechanisms including heat-shock protein inhibition. We hypothesized that combination therapy (WA + SO) would have a synergistic effect against anaplastic and papillary carcinoma cells at lower sorafenib doses.

METHODS: Human papillary (BCPAP) and anaplastic (SW1736) thyroid cancer cell lines were evaluated after treatment with SO, WA, or their combination at different doses. Proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and trypan blue exclusion; apoptosis and cell-cycle arrest was measured by flow cytometry. Western analysis confirmed apoptosis (Poly ADP ribose polymerase [PARP] and caspase-3 cleavage) and Raf inhibition. Experiments were repeated in triplicate and were evaluated statistically with significance set at a P value of less than .05.

RESULTS: The concentration of drug at which 50% of the cells are inhibited (IC_{50}) in BCPAP were 6.3 μmol/L (SO), .155 μmol/L (WA), and .055 μmol/L (IC_{50}WA + 50% IC_{50}SO), whereas in SW1736 cells the concentration was 7.6 μmol/L (SO), 2.5 μmol/L (WA), and 1.4 μmol/L (IC_{50}WA + 50% IC_{50}SO). Combination (WA + SO) at IC_{50} decreased cell viability to 19% (from 50% individually). Apoptosis levels on flow cytometry in anaplastic cells increased significantly from 0% to 2% (SO or WA alone) to 89% (combo at IC_{50}, $P < .001$). Combination therapy apoptosis (PARP cleavage and caspase-3 inactivation) and BRAF/Raf-1 down-regulation were dose-dependent starting at 50% IC_{50} levels. Cell-cycle modulation was significant with combination treatment (35% increase in G2 arrest at 50% IC_{50}SO + WA and 70% increase at 75% IC_{50}SO + WA; $P < .01$).

CONCLUSIONS: Combination therapy with sorafenib + withaferin showed synergistic efficacy in papillary and anaplastic cancers in vitro with significant induction of apoptosis. This combination achieved potent anticancer activity with lower overall doses of sorafenib, indicating a potential strategy to decrease sorafenib toxicity in future translational studies.

The authors report no conflicts of interest.

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Thyroid cancer remains the most prevalent endocrine malignancy accounting for 1% of cancers worldwide with more than 40,000 new cases diagnosed in the United States in 2010.[1] In 2011, there were an estimated 1,740 thyroid cancer deaths in the United States, representing 3.6% of all new thyroid cancer cases diagnosed.[2] Although most differentiated thyroid cancers such as papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) respond to surgery followed by radioactive iodine (RAI) therapy and thyroid hormone suppression, approximately 30% recur or develop insensitivity to RAI.[3,4] In addition, poorly differentiated subtypes including anaplastic thyroid cancer (ATC) are resistant to RAI and conventional chemotherapy carries poor long-term survival rates.[5] Surgical resection is the standard treatment for local disease or recurrence, however, a majority of patients with ATC or RAI-resistant PTC recur or progress despite optimal primary surgical resection.[6] Standard chemotherapies have limited efficacy in ATC and advanced PTC, often with response rates of less than 10% to 20% of patients and without long-term benefits. In addition, these drugs carry systemic toxicities.[5] Recent discoveries about the genetic and molecular basis of thyroid cancer have improved our understanding of this disease, leading to application of new therapies currently in phase I, II, and III human trials worldwide. Papillary thyroid cancers have been characterized by alterations of one of several kinases including rearrangements of the RET (rearranged during transfection; RET/PTC) receptor tyrosine kinase (13%–43% of cases) or point mutations in the BRAF serine/threonine kinase (29%–69% of cases).[7–9] Anaplastic thyroid cancers have the worst clinical prognosis and have mutations in BRAF (10%–35% of cases) and RAS proto-oncogenes (20%–60% of cases).[10]

Research on development of novel therapeutics in advanced thyroid cancers has focused in the past decade largely on targeted therapies such as multikinase or selective tyrosine or RET-kinase inhibitors, or inhibitors of vascular endothelial growth factor. Although these drugs showed improved efficacy over traditional cytotoxic agents in early clinical trials validating these targets, long-term efficacy is unproven and monotargeted therapies typically are limited by toxicity or eventual drug resistance. These multikinase agents act by inhibiting tumor growth and disrupting tumor vasculature through antiproliferative and proapoptotic pathways.[11] Targets of some multikinase inhibitors include Raf serine/threonine kinases, vascular endothelial growth factor receptor tyrosine kinases, and platelet-derived growth factor receptor β, and deregulation of these signaling pathways. Recent studies have shown that deregulated signaling through these receptors is seen commonly in human tumors. Sorafenib is an oral, small-molecule, tyrosine kinase inhibitor targeting vascular endothelial growth factor receptors 2 and 3, RET (including most mutant forms that have been examined), and BRAF.[12]

Natural products play a highly significant role in the drug discovery and development process and particularly in the area of cancer, in which most Food and Drug Administration–approved drugs are of natural origin. Withanolides, such as Withaferin A (WA), provide a novel mechanistic approach to thyroid cancer chemotherapy because they can interact and inhibit multiple cancer-specific pathways simultaneously without the same level of toxicity to normal cells as seen with targeted kinase inhibitors. WA is a potent inhibitor of thyroid cancer cell proliferation with concentrations of drug at which 50% of the cells are inhibited (IC_{50}) in the nanomolar concentration level in vitro.[13] Treatment with this drug leads to apoptotic cell death and has the potential to synergize with chemotherapeutics targeting other pathways. Sorafenib has been shown to have synergistic potential in other in vitro studies, therefore, it stands to reason that combining it with WA, an agent that targets multiple cancer mechanisms, might have a synergistic effect in PTCs and ATCs.[11] The aim of this study was to evaluate the synergistic potential of sorafenib combined with withaferin A in the treatment of papillary and anaplastic thyroid cancers in vitro.

Materials and Methods

Cell culture

The papillary thyroid carcinoma cell line BCPAP (with a homozygous BRAF V600E mutation) was obtained from Dr. Rebecca Schweppe (University of Colorado, Denver, CO), and the anaplastic thyroid carcinoma cell line SW1736 (with a heterozygous BRAF V600E mutation) was obtained from Dr. Nils-Erik Heldin, PhD (Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala, Sweden). BCPAP was grown in Dulbecco's modified Eagle medium (Sigma-Aldrich, St. Louis, MO) and SW1736 was grown in RPMI (Sigma-Aldrich). Both medias were supplemented with 10% fetal bovine serum (Sigma-Aldrich), sodium pyruvate, nonessential amino acids, L-glutamine, a 2-fold modified eagle media (MEM)-vitamin solution, and 1% penicillin/streptomycin (100 IU/mL/100 μg/mL, respectively; Sigma-Aldrich). Adherent monolayer cultures were maintained in T-75 culture flasks and incubated at 37°C with 5% CO_2 until they achieved 85% confluency. The cells then were trypsinized using .25% trypsin (Sigma-Aldrich) and passaged into T-75 flasks at a density of 1×106 cells. On the day of experiments, cells were trypsinized and counted via vi-Cell Counter (Beckman Coulter, Indianapolis, IN) to determine the number of viable cells.

Cell viability and proliferation assays

BCPAP and SW1736 were plated in growth media on 96-well microtiter plates at a concentration of 2×10^3 cells/well and on 6-well plates at a concentration of 2×10^4 cells/well. Cells were incubated for 24 hours at 37°C with 5% CO_2. Cells then were treated with varying concentra-