Metformin Effects on Metabolic Coupling and Tumor Growth in Oral Cavity Squamous Cell Carcinoma Coinjection Xenografts

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. Many aggressive head and neck cancers contain 2 metabolically coupled tumor compartments: a glycolytic stromal compartment with low caveolin-1 (CAV1) and high monocarboxylate transporter 4 (MCT4) expression and a highly proliferative carcinoma cell compartment with high MCT1. Metabolites are shuttled by MCTs from stroma to carcinoma to fuel tumor growth. We studied the effect of carcinoma-fibroblast coinjection and metformin administration on a mouse model of head and neck squamous cell carcinoma.

Study Design. Xenograft head and neck squamous cell carcinoma model.

Setting. Basic science laboratory.

Subjects and Methods. Oral cavity carcinoma cells were injected alone or as coinjection with human fibroblasts into nude mice to generate xenograft tumors. Tumors were excised and stained with immunohistochemistry for markers of metabolic coupling and apoptosis, including MCT1, MCT4, CAV1, and TUNEL assay (terminal deoxynucleotidyl transferase nick end labeling). Strength of staining was assessed by a pathologist or computer-assisted pathology software. Metformin was administered orally to mice to test effects on immunohistochemical markers in xenografts.

Results. Coinjection tumors were 2.8-fold larger ($P = .048$) and had 1.4-fold stronger MCT1 staining ($P = .016$) than tumors from homotypic carcinoma cell injection. Metformin decreased the size of coinjection xenograft tumors by 45% ($P = .025$). Metformin reduced MCT1 staining by 28% ($P = .05$) and increased carcinoma cell apoptosis 1.8-fold as marked by TUNEL assay ($P = .005$). Metformin did not have a significant effect on tumor size when CAV1 knockdown fibroblasts were used in coinjection.

Conclusion. Coinjection with fibroblasts increases tumor growth and metabolic coupling in oral cavity cancer xenografts. Fibroblast CAV1 expression is required for metformin to disrupt metabolic coupling and decrease xenograft size.

Keywords

tumor metabolism, metformin, metabolic coupling, head and neck cancer

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Metabolic derangement is a hallmark of cancer.1 Multicompartment metabolism and metabolic coupling, the “reverse Warburg effect,” occur in many cancers, including head and neck squamous cell carcinoma (HNSCC).2,3 Metabolic coupling is the process by which cancer cells with high mitochondrial oxidative phosphorylation (OXPHOS) metabolism metabolically reprogram nearby noncancer cells to (1) reduce OXPHOS for the cancer cells and (2) obtain metabolic by-products to fuel cancer cell growth.4 When metabolic coupling occurs, it

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leads to more aggressive tumor behavior, clinically manifested as disease recurrence and progression.\textsuperscript{3,5}

Metabolic coupling is mediated by monocarboxylate transporters (MCTs),\textsuperscript{4} which are proton-coupled symporters of pyruvate, lactate, and ketone bodies. MCT1 in cancer cell membranes acts as a catabolite importer, allowing highly proliferative cancer cells to uptake catabolites released from nearby cancer-associated fibroblasts (CAFs).\textsuperscript{6-10} MCT1, a marker of OXPHOS, is found in carcinoma cells at the leading edge of tumors in HNSCC.\textsuperscript{3} Transporter of outer mitochondrial membrane 20 (TOMM20) is a marker of functional mitochondrial mass,\textsuperscript{11} also seen in carcinoma cells in HNSCC.\textsuperscript{3} MCT4 is expressed in highly glycolytic CAFs and mediates the export of glycolysis products.\textsuperscript{3,12} CAFs have increased expression of MCT4 and decreased expression of caveolin-1 (CAV1), markers of the reverse Warburg effect, and have been associated with increased tumor aggressiveness.\textsuperscript{13}

HNSCC is often modeled with either homotypic in vitro cultures of carcinoma cells alone or homotypic in vivo injections of carcinoma cells as xenografts. Co-injection models show that the presence of fibroblasts increases primary tumor size, angiogenesis, and metastasis in some cancers.\textsuperscript{1,3,14,15} Whether co-injection affects HNSCC behavior, especially with regard to tumor metabolism, is unknown.\textsuperscript{16}

Tumor metabolism is an attractive target for anticancer therapeutics.\textsuperscript{16} Metformin inhibits mitochondrial OXPHOS and has antitumor properties\textsuperscript{17,18}, population-based studies suggest that metformin improves survival of HNSCC.\textsuperscript{19-20} Our previous window-of-opportunity clinical trial of metformin in HNSCC showed that brief treatment with metformin affects markers of tumor metabolism.\textsuperscript{21} Likewise, previous in vitro and in vivo studies showed that metformin inhibits HNSCC tumor growth.\textsuperscript{22-25} However, no tumor models have specifically focused on metabolism in the microenvironment of HNSCC or the effect of metformin therein.

Here, we sought to study markers of tumor metabolic compartmentalization in a co-injection xenograft model of HNSCC and to test the effect of metformin on our model. To our knowledge, this represents the first results of metformin treatment on a co-injection xenograft model of HNSCC.

**Materials and Methods**

**Cell Lines and Culture**

A commercially available oral tongue squamous cell carcinoma cell line, CAL27, was chosen because our previous work in HNSCC metabolism focused primarily on patients with oral cavity cancer. Fibroblasts in this study were BJ1, which are immortalized by the catalytic domain of telomerase (human telomerase reverse transcriptase [hTERT]) but not transformed. CAL27 and BJ1 were cultured in Dulbecco’s modified Eagle medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% (volume/volume) heat-inactivated fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

For coculture experiments, CAL27 and BJ1 were co-cultured in 12-well plates at a fibroblast:carcinoma ratio of 5:1. The total number of plated cells per well was 10³. For homotypic controls, a total of 10³ CAL27 or BJ1 cells were cultured alone. Cells were seeded in standard growth media that was replaced with low fetal bovine serum media (NuSerum) after 1 day. Cells were cultured for 5 days.

**Flow Cytometry**

CAL27 cells were plated in coculture with BJ1 fibroblasts that were green fluorescent protein-positive (GFP+) or plated in monoculture. After 4 days, cells were fixed in 4% paraformaldehyde, rinsed with phosphate-buffered saline (PBS), then blocked with immunofluorescence buffer (PBS, 1% bovine serum albumin, 0.1% Tween 20) for 30 minutes at room temperature. Primary anti-CAV1 (Santa Cruz Biotechnologies, Dallas, Texas), MCT1, (YenZym Antibodies, San Francisco, California), and TOMM20 (Abcam, Cambridge, Massachusetts) antibodies were incubated in immunofluorescence buffer for 1 hour at room temperature, then washed and incubated for 30 minutes at room temperature with fluorochrome-conjugated anti-rabbit and anti-mouse secondary antibodies (Fisher-Invitrogen, Carlsbad, California). Finally, cells were washed with immunofluorescence buffer. To isolate the GFP+ BJ1 cell population, cells were sorted with a GFP, PE, and APC laser. As a critical control, monocultures of CAL27 cells (GFP−) and BJ1 fibroblast cells (GFP+) were sorted in parallel.

**CAV1 Knockdown**

Cells were transfected with sh-RNA control and 2 pre-designed sh-RNAs targeting CAV1 nucleotides 383-403 (5′-GCT-GAG-GGA-GAA-GCA-AGT-GTA-3′) or 660-680 (5′-TGG-GCA-GTT-GTA-CCA-TGC-ATT-3′) of the CAV1 mRNA (NM_001753.3; Invitrogen), subcloned into the pQCXIP-GFP retroviral vector (Clontech, Mountain View, California). Finally, cells were washed with immunofluorescence buffer. To isolate the GFP+ BJ1 cell population, cells were sorted with a GFP, PE, and APC laser. As a critical control, monocultures of CAL27 cells (GFP−) and BJ1 fibroblast cells (GFP+) were sorted in parallel.

**Animals**

All animal work was approved by the Institutional Animal Care and Use Committee. Female athymic nude mice aged 4 to 6 weeks (Charles River, Wilmington, Massachusetts) were maintained under standard pathogen-free conditions. Animals were provided with sterilized chow and water. All animal handling and experiments were conducted under standards per the Institutional Animal Care and Use Committee.
Characterization of Oral Cavity Cancer CAV1

Following Institutional Review Board approval, the records of 58 consecutive patients with oral cavity squamous cell carcinoma treated surgically from 2009 to 2011 were reviewed for demographic and staging data. Formalin-fixed, paraffin-embedded (FFPE) blocks were sectioned through areas of negative margins and primary tumors to evaluate fibroblast CAV1 immunohistochemistry (IHC) staining in normal and CAFs, respectively.

Xenograft Injections and Coinjections

CAL27 and hTERT-BJ1 cells were cultured in parallel, then trypsinized and resuspended in PBS. Cells were injected via a 27-gauge needle bilaterally into the flanks of nude mice. Mice received either homotypic injections of $5 \times 10^5$ CAL27 cells in 100 µL of PBS or heterotypic injections containing $5 \times 10^5$ CAL27 cells and $1.66 \times 10^5$ hTERT-BJ1 fibroblasts (3:1 carcinoma: fibroblast ratio) in 100 µL of PBS. Each group contained 5 mice, with 2 injections in each, for a total of 10 tumors per group. Five mice also received injections of $1.66 \times 10^5$ hTERT-BJ1 fibroblasts alone, as controls.

For the CAV1-knockdown experiment, a total of 20 nude mice were injected in flanks bilaterally. Ten mice received coinjections of $5 \times 10^5$ CAL27 cells and $1.66 \times 10^5$ hTERT-BJ1 control fibroblasts. Ten mice received coinjections of $5 \times 10^5$ CAL27 cells and $1.66 \times 10^5$ hTERT-BJ1 CAV1-knockdown fibroblasts. A power calculation predicted that groups of 5 mice would have 95% power to detect xenograft size differences of 30% and a standard deviation of 20%.

Metformin Administration

For experiments including metformin, 2 groups of 5 mice each were used, for a total of 10 mice. Each mouse received identical coinjections of $5 \times 10^5$ CAL27 cells plus hTERT-BJ1 fibroblasts in each flank. Then, 1 group of 5 mice received metformin, and the other was left untreated. For the CAV1-knockdown fibroblast experiment, 5 of the 10 mice co-injected with CAL27 and control fibroblasts and 5 of the 10 mice coinjected with CAL27 and CAV1-knockdown fibroblasts were treated with metformin. Starting postinjection day 7, mice had metformin added to their drinking water at a concentration of 1 mg/mL, as previously described. Drinking water with metformin was replaced as needed, usually every 2 to 3 days. Mice drank an average of 6 mL/day. At an average mouse weight of 20 g, mice on average received 300 mg/kg/d of metformin. This dose is similar to concentrations used for intraperitoneal injections in previously described xenograft experiments.

Tumor Collection and Measurement

Tumors were measured externally by electronic calipers starting on postinjection day 7 and were then measured biweekly until postinjection day 21. Mouse weight was measured to ensure that mice were not losing weight. Calipers measured width ($x$) and height ($y$) dimensions of the tumors. On postinjection day 21, mice were sacrificed and tumors collected. Volume of excised tumors was measured by electronic calipers. Tumor volume ($V$) was calculated by the formula $V = (x^2y/2)$, as previously described.

Tumor Processing

Following extraction and measurement, xenograft tumors were preserved in 10% formalin for 2 days, then 70% ethanol. Tumors were embedded in paraffin and cross sectioned at 4-μm thickness. These FFPE tumors were used for IHC, as described next. FFPE human oral cavity squamous cell carcinoma tumors were also stained for CAV1 IHC as described here.

Immunohistochemistry

FFPE tumors were sectioned at 4 μm, dewaxed, and rehydrated through graded ethanol. Antigen retrieval was performed in 10mM citrate buffer, pH 6.0, for 10 minutes with a pressure cooker. Sections were cooled, blocked for endogenous peroxidase with 3% H2O2, and blocked for endogenous biotin with an avidin-biotin kit (Biocare Medical, Concord, California). Sections were incubated at room temperature with 10% goat serum for 30 minutes, then incubated at room temperature for 1 hour with primary antibodies for MCT1 (19-mer peptide sequence CSPDKDTEGGPKEEESPV-cooh affinity-purified rabbit antibody; YenZym Antibodies, San Francisco, California), MCT4, and CAV1 (Santa Cruz Biotechnology, Santa Cruz, California).

Primary-antibody binding was detected by biotinylated species-specific secondary antibody (Vector Labs, Burlingame, California), followed by an avidin–horseradish peroxidase conjugate (Vectastain Elite ABC Kit; Vector Labs). Immunoreactivity was revealed with 3,3’-diaminobenzidine (Liquid DAB Kit; Dako, Santa Clara, California). Sections were counterstained with hematoxylin.

TUNEL Assay

Apoptotic cells were identified with the ApopTag TdT Enzyme and ApopTag Reaction Buffer at a ratio of 20:80 (EMD Millipore, Billerica, Massachusetts) after 15 minutes of digestion with 20 µg/mL of proteinase K (Sigma-Aldrich, St Louis, Missouri), followed by anti-digoxigenin-PO (Sigma-Aldrich). TUNEL-positive cells were visualized with DAB (Dako). TUNEL slides (terminal deoxynucleotidyl transferase nick end labeling) were reviewed by a head and neck pathologist (M.T.) to ensure that areas containing carcinoma cells were quantified.

IHC Quantification

For MCT4 and CAV1, tumors were scored by a blinded pathologist. Strength of staining for MCT4 and CAV1 in carcinoma cells were quantified. For MCT4 and CAV1, tumors were scored by a blinded pathologist. Strength of staining for MCT4 and CAV1 in carcinoma cells were quantified.
reported as 0, 1+, or 2+ for fibroblasts staining <10%, 10% to 50%, and >50%, respectively.

For MCT1, a computer-assisted pathology program was used to assess strength of MCT1 staining in carcinoma cells. ImmunoMembrane is a previously described and publicly available algorithm to detect and quantify cell surface IHC markers; it was originally described for quantification of surface staining in breast cancer.\(^2^8\) Light photomicrographs (20×) were taken of 3 representative areas of tumors from each MCT1-stained xenograft tumor slide. ImmunoMembrane processes photomicrographs and produces an average score from 1 to 20, with 1 representing no staining and 20 strong staining. ImmunoMembrane scores were averaged among groups.

For TUNEL staining, quantitative analysis was performed with Aperio software (Aperio, Nussloch, Germany). Briefly, digital images were captured with Leica and Aperio scanners under 320× magnification with an average scan time of 120 seconds (compression quality, 70). A nuclear algorithm identified TUNEL-positive cells. The percentage of 3+ and 2+ nuclei was determined to accurately represent positive reactivity and generate percentage positive nuclei per area. Four tumors from each control and metformin-treated group were evaluated, and for each tumor, 6 areas encompassing the majority of viable tumor area were quantified.

Statistics
Mean tumor volume, ImmunoMembrane scores, and TUNEL staining were compared by the Student’s t test. Strength of CAV1 staining in human tumors was compared by chi-square test. Differences were considered statistically significant at \(P < .05\).

Results
Coculture
CAL27 and BJ1 cells were cultured either homotypically or in coculture. After 4 days in coculture with CAL27, BJ1 cells demonstrated decreased CAV1 expression as detected by flow cytometry (Figure 1). After 4 days in coculture with BJ1, CAL27 demonstrated increased MCT1 and TOMM20 expression as detected by flow cytometry (Figure 2).

Coinjection Effects: Tumor Size and Metabolism
Five mice received homotypic injections of CAL27 cells alone, and 5 received coinjection of CAL27 and hTERT-BJ1 fibroblasts at a carcinoma:fibroblast ratio of 3:1. All mice grew measurable tumors in both flanks. After sacrifice, coinjection tumors were 2.8-fold larger than tumors injected with CAL27 cells alone (\(P = .048\); Figure 3). Control mice injected with only hTERT-BJ1 fibroblasts did not grow xenograft tumors.

ImmunoMembrane scoring was performed for MCT1 staining in homotypic injection and coinjection tumors. MCT1 strength of staining was 1.4-fold stronger in coinjection tumors (\(P = .016\); Figure 4). MCT1 ImmunoMembrane score was 10.7 for homotypic injection tumors versus 14.5 for coinjection tumors.

Metformin Effects: Tumor Growth and Metabolism
Starting on postinjection day 7, metformin was added to the water of 5 mice that had received coinjections of CAL27 and hTERT-BJ1 fibroblasts. At the time of tumor sacrifice, xenograft tumors treated with metformin had a 45% smaller volume than those from untreated mice (\(P = .025\); Figure 5).

ImmunoMembrane scoring was performed for MCT1-stained tumors from untreated and metformin-treated mice. MCT1 strength of staining was 28% lower in metformin-treated tumors (\(P = .050\); Figure 6A-C). Average MCT1 ImmunoMembrane score was 13.2 for control tumors versus 10.9 for metformin-treated tumors. Metformin-treated tumors had a 1.8-fold more apoptotic nuclei as measured by TUNEL assay (\(P = .005\); Figure 6D-F). TUNEL score was 3.3% positive nuclei per field for control tumors versus 6.1% for metformin-treated tumors.

Stromal CAV1 staining was negative (0) in 3 of 4 control tumors and was 1+ or 2+ in 3 of 4 metformin-treated tumors.
tumors. There was no difference in MCT4 staining between groups (data not reported).

CAV1-Knockdown Fibroblasts

Starting on postinjection day 7, metformin was added to the water of 5 mice that received coinjections with control fibroblasts and 5 mice that received coinjection with CAV1-knockdown fibroblasts. In control fibroblast coinjection tumors, metformin reduced tumor volume by 61% ($P < .001$; Figure 7A). In CAV1-knockdown fibroblast coinjection tumors, metformin reduced average tumor volume by 34%, but this difference was not statistically significant ($P = .38$; Figure 7B).

CAV1 Staining in Human Oral Cavity Tumors

The records of 58 surgically treated patients with oral cavity squamous cell carcinoma were reviewed, and their tumors and normal mucosa were evaluated for strength of fibroblast CAV1 staining. Clinical and pathologic data are reported in Table 1. In normal stroma, 25 patients (43%) had $2+$ fibroblast CAV1 staining; 33 (57%) had $1+$ CAV1 staining; and none had $0+$ CAV1 staining. At the leading edge of tumors, 11 patients (19%) had $2+$ fibroblast CAV1 staining; 30 (52%) had $1+$ staining; and 17 (29%) had $0+$ staining (Table 2, Figure 8). On comparison by chi-square test, the fibroblasts at the tumor-stroma interface had significantly less CAV1 staining ($P < .001$), consistent with a loss of CAV1 expression in CAFs.

Discussion

Multicompartment tumor metabolism and metabolic coupling are markers of aggressive tumor behavior in head and neck cancer. Multicompartment tumor metabolism and metabolic coupling are markers of aggressive tumor behavior in head and neck cancer. Multicompartment tumor metabolism and metabolic coupling are markers of aggressive tumor behavior in head and neck cancer. Multicompartment tumor metabolism and metabolic coupling are markers of aggressive tumor behavior in head and neck cancer. Here, we show that coinjection of cancer cells with fibroblasts favors tumor xenograft growth and increases markers of metabolic compartmentalization in a mouse model of HNSCC. Orally administered metformin inhibits tumor growth and improves markers of metabolic coupling but does not effectively inhibit tumor growth in the absence of fibroblast CAV1.

Interactions between cancer cells and CAFs have major impact on tumor aggressiveness. In our HNSCC model, we show that coinjection of cancer cells with fibroblasts increases tumor xenograft growth when compared with homotypic injection of cancer cells. Moreover, the presence of fibroblasts induces metabolic compartmentalization, as
shown by an increased expression of MCT1 in cancer cells. In HNSCC, MCT1 is a marker of high metabolism in mitochondria and metabolic coupling between carcinoma and stromal cells, and it is found in the highly proliferative leading edge of tumors.\(^3,29\)

Metformin is a mitochondrial OXPHOS inhibitor and has been shown to decrease tumor growth in head and neck homotypic xenograft injections.\(^{22-24}\) Our experiments show that metformin also inhibits tumor growth in the setting of carcinoma-fibroblast coinjection, where tumors behave more aggressively and have increased markers of metabolic coupling. Metformin decreased expression of carcinoma MCT1, suggesting a decrease in metabolic coupling between tumor and stromal compartments. Metformin also increased

Figure 3. Tumor volume of homotypic CAL27 injection and coinjection with fibroblasts. (A) Xenograft tumor volume was significantly larger with coinjection versus carcinoma injection alone. (B) Top row of tumors is homotypic injection; bottom row, coinjection. Values presented as mean ± SEM.

Figure 4. MCT1 expression quantification of homotypic (A) CAL27 and (B) coinjection CAL27 + Bj1 tumors. (C) Average intensity of MCT1 staining in tumor groups. Note higher MCT1 staining intensity in coinjection tumors. MCT1, monocarboxylate transporter 1. Values are presented as mean ± SEM.
fibroblast CAV1 expression in tumors generated by coinjection of carcinoma cells and fibroblasts, suggesting that metformin has effects on both types of cells involved in metabolic coupling. In addition to changes in markers of metabolic coupling, metformin increased carcinoma cell apoptosis. These increases in fibroblast CAV1 and cancer cell apoptosis were reported in our recently published clinical trial with oral administration of metformin to patients with HNSCC. Whether increased apoptosis is a direct effect of metformin, a by-product of impaired metabolic coupling, or it occurs by another mechanism is still unknown.

One limitation of quantifying apoptosis with TUNEL is that carcinoma cells constitute a heterogeneous population of metabolic phenotypes. It is difficult to determine whether metformin induces apoptosis only in cells undergoing OXPHOS, only in glycolytic cells, or in both. Metformin has also been shown to increase intracellular lactate in HNSCC, future studies may quantify intracellular lactate in tumor xenografts.
Some insight into metformin’s antitumor mechanism was revealed in our in vivo experiment involving CAV1-knockdown fibroblasts. CAV1 is normally highly expressed in fibroblasts not in proximity to cancer cells but is downregulated in CAFs in some cancers. Low fibroblast CAV1 expression is associated with poor outcomes in melanoma, prostate, breast, stomach, pancreatic, and esophageal squamous cell carcinoma. Metformin restores fibroblast CAV1 in HNSCC, and previous work in non–small cell lung carcinoma suggested that CAV1 is necessary for metformin’s antitumor effect. Here, metformin did not have a significant impact on tumor growth when CAV1-knockdown fibroblasts were used in xenograft coinjections. Future studies may include fibroblasts with CAV1 overexpression to determine whether carcinoma cells can effectively “couple” with CAV1-expressing fibroblasts and whether metformin’s effect might be enhanced by CAV1 overexpression.

The importance of CAV1 downregulation in CAFs is highlighted by the pattern of CAV1 staining in human oral cavity tumors: CAFs had significantly less CAV1 expression when compared with normal fibroblasts. While IHC does not allow for direct comparison of CAV1 levels in human tumors and xenograft tumors, the patterns of staining are the same, with loss of CAV1 expression in a CAF compartment (Figure 8).

Metformin’s ability to affect metabolic coupling and tumor growth in our xenograft experiment is a promising result in establishing metabolic targets for anticancer agents. Metformin decreased carcinoma expression of MCT1 and decreased tumor growth. Alpha-cyano-4-hydroxycinnamate and AZD3965, both MCT1 inhibitors, have been shown to inhibit cancer growth in vitro and in vivo in glioblastoma, non–small cell lung cancer, cervical squamous cell carcinoma, and colorectal adenocarcinoma. No clinical trials of MCT1 inhibitors have been published to date, nor have effects of direct MCT1 inhibition in HNSCC.

Table 1. Demographic and Staging Information for 58 Patients With Oral Cavity Squamous Cell Carcinoma Treated Surgically.

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Patients, n (%)</th>
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<td>62.8 (33-91)</td>
<td>23 (39)</td>
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Table 2. Strength of Immunohistochemistry Staining as Scored by Blinded Pathologist for Normal Fibroblasts and CAFs.*

<table>
<thead>
<tr>
<th>Caveolin-1 Score</th>
<th>Normal Fibroblasts (n = 58)</th>
<th>CAFs (n = 58)</th>
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<tr>
<td>0</td>
<td>0 (0)</td>
<td>17 (29)</td>
</tr>
<tr>
<td>1</td>
<td>33 (57)</td>
<td>30 (52)</td>
</tr>
<tr>
<td>2</td>
<td>25 (43)</td>
<td>11 (19)</td>
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Abbreviation: CAF, cancer-associated fibroblast.

*Values are presented as n (%). Groups were significantly different on chi-square test (P < .001).
Altered tumor metabolism is an attractive anticancer target and, when combined with appropriate tumor characterization, may improve survival outcomes among cancer patients. Our results provide further evidence regarding the importance of metabolic tumor coupling in HNSCC. We provide the first evidence of the impact of fibroblasts on metabolic reprogramming of HNSCC in a mouse model (Figure 9). Moreover, we demonstrate metformin’s ability to affect both metabolic coupling and tumor growth, and we identify fibroblast CAV1 as a mediator of metformin’s effects. Our study is limited by the use of nude instead of immunocompetent mice, relatively small numbers of xenografts, and the use of IHC, which is not strictly quantitative. Our results contribute to further understanding the metabolic profile of HNSCC, aiding the development of novel anticancer treatments targeting the cancer microenvironment.

Acknowledgments
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17. Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford LG. Repurposing metformin for the prevention of cancer and cancer recurrence [published online August 3, 2017]. Diabetologia. doi:10.1007/s00125-017-4372-6


