Defects in Cell Growth Regulation by C_{18:0}-Ceramide and Longevity Assurance Gene 1 in Human Head and Neck Squamous Cell Carcinomas

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In this study, endogenous long chain ceramides were measured in 32 human head and neck squamous cell carcinoma (HNSCC) and 10 nonsquamous head and neck carcinoma tumor tissues, as compared with adja-
cent noncancerous tissues, by liquid chromatography/mass spectroscopy. Interestingly, only one specific cer-
amide, C_{18:0}-ceramide, was selectively down-regulated in the majority of HNSCC tumor tissues. On the other hand, in nonsquamous tumor tissues, this selectivity for C_{18:0}-ceramide was not detected. These data suggested the hypotheses that decreased levels of C_{18:0}-ceramide might impart a growth advantage to HNSCC cells and that increased generation of C_{18:0}-ceramide may be in-
volved in the inhibition of growth. These roles were examined by reconstitution of C_{18:0}-ceramide at physio-
logically relevant concentrations in UM-SCC-22A cells (squamous cell carcinoma of hypopharynx) via overex-
pression of mammalian upstream regulator of growth and differentiation factor 1 (mUOG1), which has been shown to specifically induce the generation of C_{18:0}-ceramide. Liquid chromatography/mass spectroscopy analysis showed that overexpression of the mLAG1/mUOG1 resulted in increased levels of only C_{18:0}-cer-
amide by ~2-fold, i.e. concentrations similar to those of normal head and neck tissues. Importantly, increased generation of C_{18:0}-ceramide by mLAG1/mUOG1 inhibited cell growth (~70–80%), which mechanistically involved the modulation of telomerase activity and induction of apoptotic cell death by mitochondrial dysfunction. In conclusion, this study demonstrates, for the first time, a biological role for LAG1 and C_{18:0}-ceramide in the regulation of growth of HNSCC.

Squamous cell carcinoma of the head and neck (HNSCC), one of the six most common cancers in the world, is associated with poor survival and high mortality rates. Global occurrence rate of HNSCC is estimated to be ~5% of the total malignan-
cies in the adult population, and in the United States there are ~40,000 annual cases of HNSCC (1–3). Despite advances in treatment, including surgery, radiation, and chemotherapy, survival statistics of patients with this disease have not im-
proved significantly in decades (3, 4). Although p53, cyclin D1, K-Ras, Rb, and telomerase have been identified as prognostic markers for HNSCC (5), delineation of pathogenic mechanisms is required for understanding the biology of these tumors, for designing optimized therapy, and for identification of specific diagnostic and prognostic markers.

The bioactive sphingolipid ceramide, an emerging tumor suppressor lipid, mediates anti-proliferative responses such as apoptosis, cell cycle arrest, and senescence (6, 7). Our recent studies have shown that ceramide is one of the upstream reg-
ulators of telomerase activity (8–10) and that the regulation of telomerase by ceramide involves the inactivation of c-Myc trans-
scription factor via increased ubiquitin/proteasome function for its rapid proteolysis (11). It is well established that telomerase is active in ~80–90% of the tumor tissues of the HNSCCs, whereas it is not active in normal head and neck tissues (12–15). These results suggested to us that endogenous levels of ceramide may also be misregulated in HNSCC.

Endogenous ceramide levels can be altered by various mechanisms, including de novo synthesis of ceramide or ac-
tivation of sphingomyelinas, which can regulate cell growth (16). One of the key enzymes of the de novo pathway is (dihydro)ceramide synthase, and in Saccharomyces cerevisiae longevity assurance gene 1 (LAG1) and its homologue Lac1 were identified as components of ceramide synthase activity (17, 18). Recent studies have shown that one of the mouse homologues of LAG1, known as upstream of growth and differentiation factor 1 (mUOG1 or mLAG1) selectively reg-
ulates the synthesis of stearoyl (C_{18:0})-containing sphingolip-
ids, including C_{18:0}-ceramide (19). The human homologue of the mLAG1/mUOG1 (hLAG1, hLAG1Hs, or LASS1) has re-
cently been identified and has >80% amino acid homology with mLAG1 (20, 21). Subsequently, two additional homo-
carcinoma; LAG1, longevity assurance gene 1; mLAG1, mouse LAG1; mUOG1, mammalian upstream regulator of growth and differentiation factor 1; hLAG1, human LAG1; LC/MS, liquid chromatography/mass spectroscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-
lium bromide; Q-PCR, quantitative real time PCR; RT, reverse trans-
scription; TRAP, telomere repeat amplification protocol.
logue of mammalian LAG1 family members, *trh1* and *trh4*, were identified to regulate ceramide synthesis using different fatty acyl CoA donors (22). Overexpression of *trh1* increased mainly stearic acid and arachidic acid-containing sphingolipids, and *trh4* overexpression elevated mainly palmitic acid-containing sphingolipids (22).

Because the clinical relevance of endogenous ceramide and its roles in the regulation of cell growth in HNSCC has not been established previously, this study first focused on examining the levels of endogenous ceramide in clinical samples obtained from patients with HNSCC. Unexpectedly, the data demonstrated that only C18-ceramide is down-regulated significantly in the majority of the HNSCC when compared with their adjacent normal tissues. However, in nonsquamous tumors, this selective down-regulation of C18-ceramide was not detected, suggesting a specific role for C18-ceramide in the pathogenesis/progression of HNSCC. Further evidence is presented to establish the role of LAG1/C18-ceramide in the inhibition of growth, modulation of telomerase activity, and induction of apoptosis in HNSCC cells *in vitro*. To our knowledge, this is the first study demonstrating a biological role for LAG1 and C18-ceramide in the regulation of growth of HNSCC.

**EXPERIMENTAL PROCEDURES**

*Tissue Samples and Statistical Analysis—*Tumors and their paired adjacent normal tissues of HNSCC and nonsquamous head and neck cancer patients were obtained from the Tumor Bank at the Hollings Cancer Center (Medical University of South Carolina) with the permission of the Institutional Review Board. Normal adjacent tissues, which were mucosal tissues that were not grossly or histologically cancerous or precancerous, were excised at least 1 cm away from the main tumor mass of the patients. Some of the patients (HNSCC patients 1–17) have tissues from their tumor mass and from their normal adjacent part, which were referred as “paired” samples, and some patients (HNSCC patients 18–32) have only their tumor tissues without normal adjacent tissues. The type of disease and the levels of

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**Fig. 1. Analysis of ceramide levels in tumor versus normal tissues of patients with HNSCC.** Levels of ceramide in tumors and their paired adjacent normal tissues obtained from patients with HNSCC were measured by LC/MS as described under “Experimental Procedures.” First, total ceramide levels (A) and ceramide species (B) in 17 paired samples of HNSCC tissues were analyzed. The total levels of only C18-ceramide was lower in tumor as compared with normal tissues (B). The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.
C<sub>18</sub>-ceramide in tumors as compared with normal tissues are summarized in Tables I and II. Ceramide measurements in these samples were performed by LC/MS as described below. Statistical analyses of the results were performed using Student’s t test.

**Measurement of Ceramide Levels Using High Performance LC/MS**—The cellular levels of endogenous ceramides were measured using normal phase high performance liquid chromatography coupled to atmospheric pressure chemical ionization-mass spectrometry (LC/MS) as described previously (9). The ceramide levels were normalized to total protein levels (0.5 mg of protein/sample).

**Cell Lines and Culture Conditions**—Human head and neck cancer cell lines UM-SCC-1 (SCC of retromolar trigone/floor of the mouth) and UM-SCC-22A (SCC of hypopharynx) cells (23) were obtained from Dr. Thomas Carey at the Department of Otolaryngology/Head and Neck Surgery, University of Michigan. The cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and 1% penicillin/streptomycin at 37 °C in 5% CO<sub>2</sub>.

**Detection of Growth Inhibition by MTT and Trypan Blue Assays**—The concentrations of agents that inhibited cell growth by 50% (IC<sub>50</sub>) were determined from cell survival plots obtained by MTT or trypan blue assays as described (8). Triplicate wells were used for each treatment.

**Plasmids and Transfections**—The complete cDNA (1.4 kb) for mammalian UOG1 (mlAG1/mlUOG1) was cloned in the pCMVexSVneo plasmid and used for transfections of UM-SCC-22A and UM-SCC-1 cells using an Effectene transfection kit (Qiagen) as described previously (8).

**Quantitative Real Time (Q)-PCR and Conventional Semi-quantitative Reverse Transcription (RT)-PCR**—One μg of total RNA, isolated using an RNA isolation kit (Qiagen), was used in reverse transcription reactions as described (8). The resulting total cDNA was then used in the Q-PCR or RT-PCR as described previously (8, 16). The mRNA levels of β-actin and rRNA were used as internal controls.

**Measurement of Telomerase Activity**—Telomerase activity in cell extracts was measured by the PCR-based telomere repeat amplification protocol (TRAP) using TRAPEZE kit (Intergen, Gaithersburg, MD) which includes a 36-bp internal control to allow quantification of activity as described (8–10).

**Analysis of Mitochondrial Membrane Potential**—The collapse of an electrochemical gradient across the mitochondrial membrane during apoptosis was measured using a JC-1 mitochondrial membrane potential detection kit (Cell Technology) by flow cytometry as described by the manufacturer. This kit uses a unique cationic dye, JC-1, to signal the loss of the mitochondrial membrane potential. In healthy cells, the dye accumulates in the mitochondria as aggregates, which become fluorescent red. In apoptotic cells, the mitochondrial potential collapses, and the JC-1 cannot accumulate within the mitochondria and remains in the cytoplasm as a green fluorescent monomeric form. These different forms of JC-1 were then detected by flow cytometry as described by the manufacturer.

**RESULTS**

**Analysis of Ceramide Levels in HNSCC Tumor Tissues**—The levels of endogenous ceramides in 17 pairs of HNSCC and 10 pairs of nonsquamous head and neck tumor tissues were measured and compared with their paired adjacent noncancerous tissues using LC/MS as described. Unexpectedly, as shown in Fig 1A, the results showed that the total ceramide levels were higher in the HNSCC tumor tissues as compared with their noncancerous tissues (2393 and 1505 pmol/0.5 mg protein, respectively). However, when individual ceramide species were examined in these paired samples (n = 17), the results demonstrated that only C<sub>18</sub>:0-ceramide was ~50% lower in HNSCC tumor tissues as compared with their noncancerous counterparts (103 versus 196 pmol/0.5 mg protein), whereas the levels of all the other ceramides were generally higher in HNSCC tumors than their controls (Fig 1B). When the levels of C<sub>18</sub>:0-ceramide were further evaluated in each patient sample, the results showed that 12 of 17 patients (70% of the patients, p < 0.01) had significantly lower levels of C<sub>18</sub>:0-ceramide in their tumor tissues compared with their adjacent noncancerous tissues (Fig. 2A and Table I, patients 1–17).
To investigate whether decreased levels of C18-32-eracamide are due to altered expression of hLAG1, a human homologue of mLAG1/mUOG1 that has been shown to specifically increase C18-32-eracamide generation, total RNA was isolated initially from normal and HNSCC tumor tissues of the patient 6, and then mRNA levels of hLAG1 were examined by RT-PCR and Q-PCR (Fig. 2, B and C, respectively). The results showed that hLAG1 mRNA expression was significantly decreased in the tumor as compared with its adjacent normal tissue (Fig. 2, lanes 3 and 2, respectively, and C), and this correlated with the decreased levels of C18-32-eracamide in the tumor tissue of this patient (Fig. 2A). However, mRNA levels of hLAG1 were similar in both the normal and tumor tissues of patient 9 (Fig. 2D), whose C18-32-eracamide levels were also comparable in these tissues. These data indicate that C18-32-eracamide levels might be regulated by the expression of LAG1 at the mRNA level in HNSCC; however, this needs to be determined in a larger cohort of patient samples, when they are available.

The levels of C18,0-eracamide in noncancerous tissues obtained from patients with HNSCC were similar (−11.5 pmol/500 μg protein/sample with a standard deviation of ±4). Therefore, the average level of C18,0-eracamide of these noncancerous tissues was used in the examination of ceramide levels in 15 additional HNSCC tumor tissues for whom matched noncancerous tissues were not available (Fig. 3A and Table I, patients 18–32). The results of the LC/MS analysis showed that 7 of 15 tumor tissues exhibited significantly lower levels of C18,0-eracamide as compared with controls (Fig. 3A and Table I, patients 18–32). Taken together, these results demonstrate that C18,0-eracamide levels are significantly lower in approximately 19 of 32 (~60% of the patients, p < 0.01) HNSCC tumor tissues (Table I). In addition, the level of C18,0-eracamide in the serum of HNSCC patients were similar to that of their noncancerous head and neck tissues (the average value of C18,0-eracamide in the serum of these patients was −10.5 pmol/500 μg protein), suggesting that the amount of this ceramide in the tumor site, and not in the whole blood/serum, may be important for its regulatory roles in the pathogenesis/progression of the HNSCC.

Interestingly, in nonsquamous tissues (n = 10), a selective decrease in the levels of C18-32-eracamide was not detected. Instead, lower levels of C18-32-eracamide (lower in 6 of 10 patients; p < 0.05), were observed in the tumor tissues as compared with their adjacent noncancerous tissues (Fig. 3, B and C, respectively, and Table II). Similar results were also observed in nonsquamous cell lung carcinomas, in which significantly reduced levels of C18, C18, and C24-eracamides were detected in the majority of the tumors (~80%) as compared with their adjacent normal lung tissues (n = 10, p < 0.001, data not shown).

Taken together, these results suggest an important and novel role for C18-32-eracamide and LAG1 in the pathogenesis/progression of the HNSCC, whereas in nonsquamous tumors (head and neck, and lung), decreased levels of major ceramides such as C18, C18, and C24-eracamides appear to be important.

### Analysis of the Role of C18,0-eracamide by Overexpression of the mLAG1/mUOG1 cDNA in the Regulation of Cell Growth in UM-SCC-22A Cells—Decreased levels of C18,0-eracamide in the majority of the HNSCC tumor tissues compared with their adjacent noncancerous tissues suggested the hypothesis that although altered levels of C18-32-eracamide might play important roles in the pathogenesis/progression of HNSCC, its increased generation/accumulation might regulate the growth of HNSCC cells. To test this hypothesis, mLAG1/mUOG1 was overexpressed in the human HNSCC cell line UM-SCC-22A (SCC of the hypopharynx) as described under “Experimental Procedures.”

Overexpression of mLAG1/mUOG1 was confirmed by Q-PCR (Fig. 4A). This resulted in an increase selectively in the levels of C18-32-eracamide from ~6 to 11.5 pmol/0.5 μg protein (Fig. 4B), which are similar to the levels detected in normal head and neck tissues (Fig. 2A). Interestingly, the levels of other major ceramides such as C14, C24, and dihydro-C18-eracamides (Fig. 4C, left panel) and C18-32-eracamide (Fig. 4C, right panel) were decreased significantly, when compared with vector-transfected controls.

The Effects of mLAG1/mUOG1 on the Regulation of Growth, Telomerase Activity, and Apoptosis—The effects of overexpression of mLAG1/mUOG1 on cell growth were examined using the trypan blue exclusion assay. The results demonstrated that mLAG1/mUOG1 expression caused ~82% inhibition of cell growth as compared with controls (Fig. 5A). Similar results were also obtained using MTT assays, in which overexpression of mLAG1/mUOG1 resulted in ~80% decrease in cell growth in these cells (data not shown).

Because ceramide is known to mediate the inhibition of telomerase in various human cancer cells (8, 9) and because telomerase activity has been detected in the majority of HNSCC tumors and not in normal head and neck tissues (12–15), the role of C18-32-eracamide in the inhibition of telomerase was examined in UM-SCC-22A cells. The data showed that increased generation of C18-32-eracamide by mLAG1/mUOG1 re-
resulted in a significant inhibition of telomerase activity (~50%) when compared with controls (Fig. 5B, lanes 2 and 1, respectively). Interestingly, additional data using Q-PCR indicated that the inhibition of telomerase activity by LAG1/C18-ceramide may not be due to decreased mRNA expression of its catalytic human telomerase reverse transcriptase (hTERT) (shown in Fig. 5C) or human telomerase RNA (hTR) subunits (data not shown), suggesting a post-transcriptional regulation.

In addition, analysis of cell cycle by flow cytometry showed that mLAG1/mUOG1 significantly increased the number of apoptotic cells in the sub-G0/G1, whereas there were no significant changes in the cell cycle profiles (Fig. 5D). To further evaluate whether mLAG1/mUOG1/C18-ceramide-induced apoptosis involves the mitochondrial death pathway, analysis of mitochondrial potential using JC-1 by flow cytometry was performed as described under “Experimental Procedures.” The data showed that mLAG1/mUOG1 expression resulted in a significant loss of mitochondrial membrane potential, as determined by increased accumulation of JC-1 (39%) as green monomers in the cytoplasm (Fig. 5E), suggesting a role for LAG1/C18-ceramide in mediating the mitochondrial death pathway.

To determine whether the role of increased generation of C18:0-ceramide by mLAG1/mUOG1 in the inhibition of growth is cell line-specific, mLAG1/mUOG1 was expressed in another human HNSCC cancer cell line, UM-SCC-1 (squamous cell carcinoma of the floor of the mouth), and the levels of ceramides and its effects on growth were determined using LC/MS and MTT assays, respectively. Expression of mLAG1/mUOG1, confirmed by RT-PCR (Fig. 6A, right panel), resulted in a significan-
cant increase (~6-fold) in the generation of C_{18:0}-ceramide (Fig. 6, A and B), whereas the levels of other ceramides, especially C_{16}-ceramide, were significantly reduced as compared with controls (Fig. 6B, upper and lower panels). Increased levels of C_{18:0}-ceramide in response to mLAG1/mUOG1 overexpression were also accompanied by a significant inhibition of cell growth (~70%) in these cell lines (Fig. 6C). Thus, these results strongly demonstrate that inhibition of growth by mLAG1/mUOG1 and C_{18:0}-ceramide is not cell line-specific but rather can be detected in other human HNSCC cell lines.

**DISCUSSION**

The results presented in this study demonstrate that decreased levels of C_{18:0}-ceramide might play a role in the pathogenesis/progression of the HNSCC; on the other hand, in nonsquamous tissues, this selectivity for C_{18:0}-ceramide was not detected. These results suggest that decreased levels of C_{18:0}-ceramide may impart a growth advantage to cancer cells, whereas increased generation/accumulation of C_{18:0}-ceramide may lead to inhibition of growth by HNSCC cells. This was further supported by the data showing that increased generation of C_{18:0}-ceramide via overexpression of mLAG1/mUOG1 results in the inhibition of growth, which involves the modulation of telomerase activity and induction of apoptosis in HNSCC cells. Taken together, these results provide evidence for the role of LAG1 and C_{18:0}-ceramide in the regulation of telomerase and apoptosis in HNSCC cells.

The anti-proliferative roles of endogenous ceramide have been demonstrated in various human cancer cells previously (reviewed in Ref. 7). A recent study demonstrated that the total ceramide levels are inversely correlated with malignant progression and poor prognosis of astrocytomas (24). The results from the present study support these conclusions, and they further provide additional information that the levels of specific ceramide species, such as C_{18:0}-ceramide, and not total ceramide levels might also be important in the pathogenesis and regulation of cell growth in some carcinomas such as HNSCC. Therefore, the identification of immediate downstream targets of C_{18:0}-ceramide, which are involved in the inhibition of cell growth in HNSCC cells, is extremely important and of great interest in our laboratory. In fact, the present study shows that overexpression of mLAG1/mUOG1 results in the inhibition of telomerase activity, which has been shown to be elevated in the majority of HNSCC tumor tissues and not in the normal tissues (12–15). Thus, these results support that telomerase is one of the cancer-specific targets of endogenous ceramide. Interestingly, inhibition of telomerase in response to C_{16}-ceramide or daunorubicin was linked to the generation of endogenous C_{16}- and C_{24}-ceramides in the A549 human lung
adenocarcinoma cells (8). This inhibition, however, was mainly due to decreased mRNA expression of human telomerase reverse transcriptase and correlated with cell cycle arrest but not apoptosis. However, in HNSCC cells, the inhibition of telomerase activity by LAG1/C18-ceramide pathway appears to be at the post-transcriptional level and correlates with apoptosis. Thus, these data suggest that specific ceramides might have distinct functions in the regulation of telomerase and apoptosis, and these functions might be cell type- or tissue-specific.

LAG1 was discovered in yeast, and it has been shown that deletion of LAG1 extends the life span in yeast (17, 25, 26). The mammalian homologues of yeast LAG1 were later identified in mice, called mammalian upstream of growth and differentiation 1, mUOG1 (19), and in human called human LAG1 (also referred to as LASS1, human longevity assurance homologue 1 of yeast LAG1) (17, 25). The mUOG1 and human LAG1 have been shown to have >80% amino acid homology (20). Further studies showed that the biological activities of LAG member genes encode essential components of acyl-CoA-dependent (dihydro)-ceramide synthase (18). Interestingly, mUOG1 has been shown to be involved in the synthesis of C_{18} (stearic acid) containing sphingolipids, including C_{18:0}-ceramide (19). However, the role for these LAG member proteins in the regulation of cell growth in mammals has not been shown previously. Therefore, our results presented here demonstrate that LAG1/C_{18}-ceramide is involved in the regulation of cell growth in UM-SCC-22A and UM-SCC-1 cells.

Interestingly, our data demonstrated that the levels of C_{16}-ceramide is significantly up-regulated in HNSCC tumors as compared with their normal adjacent tissues. In parallel with these findings, the level of C_{16}-ceramide is greatly reduced upon mLAG1/mUOG1 overexpression and increased generation of C_{18}-ceramide in HNSCC cells (see Figs. 4 and 6). These results are surprising, because the role for increased C_{16}-ceramide in apoptosis upon induction by IgM via de novo pathway in Jurkat cells, or in cell cycle arrest and the inhibition of telomerase activity in the A549 nonsquamous lung cancer cells, has been demonstrated previously (10, 27). Therefore, determining the possible relationship, if any, between increased levels of C_{16}-ceramide in HNSCC tumors and its decreased levels in response to mLAG1/mUOG1 expression in HNSCC cell lines is important and needs to be further explored. In light of this, specific downstream targets and subcellular localization compartmentalization of these ceramides (C_{16} and C_{18})...
showed decreased levels of C18-ceramide selectively in the cell lines (34). It is also interesting that our results specifically distributions of sphingolipids were also different among these amounts and types of sphingolipids, and the fatty acyl chain shown that different glioma cell lines had differences in the lines have been performed previously (34). The results have metabolomic profiling of sphingolipids in human glioma cell pathogenesis of various carcinomas including HNSCC. In fact, sample, is a powerful tool to discover the roles for specific can identify the levels of different ceramide species in a given results also indicate that the use of LC/MS (9, 32, 33), which clinical relevance of ceramide in HNSCC is still unknown. The role of C18-ceramide in HNSCC is still unknown. The role of C18-ceramide in the inhibition of growth of various cancer cells (28–30). Treatment of the Tu138 human head and neck squamous cell carcinoma cells with paclitaxel and C6-ceramide in combination synergistically inhibited cell growth (31), demonstrating a role for ceramide in the regulation of growth or apoptosis in various different types of cancer cells, and this is of great interest in our laboratories.

It is also known that increased ceramide generation in response to various stress stimuli including radiation and chemotherapeutic agents can result in the inhibition of growth of various cancer cells (28–30). Treatment of the Tu138 human head and neck squamous cell carcinoma cells with paclitaxel and C6-ceramide in combination synergistically inhibited cell growth (31), demonstrating a role for ceramide in the treatment of head and neck cancer cells in vitro. However, the clinical relevance of ceramide in HNSCC is still unknown. The results also indicate that the use of LC/MS (9, 32, 33), which can identify the levels of different ceramide species in a given sample, is a powerful tool to discover the roles for specific ceramide species (with different fatty acid chain length) in the pathogenesis of various carcinomas including HNSCC. In fact, metabolomic profiling of sphingolipids in human glioma cell lines have been performed previously (34). The results have shown that different glioma cell lines had differences in the amounts and types of sphingolipids, and the fatty acyl chain distributions of sphingolipids were also different among these cell lines (34). It is also interesting that our results specifically showed decreased levels of C18-ceramide selectively in the squamous cell carcinoma of the head and neck tissues, whereas lower levels of C18 and C16-ceramides were detected in nonsquamous head and neck tumor tissues. The association of lower C18-ceramides, if any, with prognosis and/or survival in these patients with HNSCC could not be examined because of the high grades of the tumors and the lack of follow up information about the patients for a longer period of time. The clinical importance of C18-ceramide in overall survival and prognosis in HNSCC needs to be determined in studies with a larger cohort of patients.

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FIG. 6. The role of mLAG1/mUOG1 in the inhibition of growth in UM-SCC-1 cells. The role of C18-ceramide in the inhibition of growth was examined by overexpression of mLAG1/mUOG1 in UM-SCC-1 cells. The overexpression of mLAG1/mUOG1 after transient transfections for 48 h (lane 2) were confirmed by RT-PCR (A, right panel), which resulted in increased generation of only C18-ceramide (A and B) and not other ceramide species (B, upper and lower panels). The effects of increased C18-ceramide on the inhibition of cell growth via expression of mLAG1/mUOG1 (C) was determined using MTT assays. The results shown are representative of two independent experiments. The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs.
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