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BRIEF COMMUNICATION

Music Is Capable of Inducing Changes in Gene Expression in Gastric Cancer Cells

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Abstract

Purpose Music has recognized beneficial effects on cancer patients; however, very little is known about the molecular processes which produce these benefits. The aim of this work was to evaluate the effect of music on proliferation and gene expression in gastric cancer cells.

Methods AGS gastric cancer cells were exposed to metal and classical music, and subsequently cell proliferation and expression of genes associated with apoptosis and cell-cycle control were evaluated.

Results Proliferation of AGS cells increased when exposed to metal music, but not when exposed to classical music. Gene expression of caspase-3 and 8 and cyclin B1 increased in response to both musical genres; classical music repressed the expression of p53, and metal music repressed the expression of PUMA.

Conclusions This is the first study to demonstrate music as a modulator of gene expression in a cancer cell line. Additional experiments are required to better understand the mechanisms of how different musical genres can induce changes in gene expression.

Keywords Gastric cancer · Music · Apoptosis · Gene expression

Introduction

Much has been written both in informal circles and in the scientific community about the beneficial effects of music on cancer patients. In the same respect, diverse studies suggest that music may be useful in medical care, alleviating nociception and stress for cancer patients [1]. A recent meta-analysis found 25 articles, comprising a total of 1784 patients, indicating that music has somatic and psychological benefits on patients in different stages of cancer, especially for relieving anxiety and pain, and improving mood [2]. Another meta-analysis reviewing 52 trials with a total of 3731 participants, found that musical intervention might have a beneficial effect on anxiety in cancer patients [3].

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Recently, scientists have found that positive emotional effects were induced by a vibroacoustic TAO-tuned sound-bed in cancer patients compared to those who did not receive the musical treatment. The experimental group experienced enhanced inner balance, vitality, and vigilance in response to the vibroacoustic treatment, which was not observed in the control group [4]. Diverse studies suggest that music regulates social hormones such as cortisol, testosterone, estrogen, oxytocin, and arginine vasopressin, which could explain these results [5].

What then, is the molecular effect of music in cancer patients? One study found that patients who participated in choirs had decreased cortisol and increased cytokine levels, including IL2, IL4, IFNγ, and TNFα. In particular, the cytokine TNF-α is involved in tumorigenesis via cellular transformation, angiogenesis, and metastasis [6].

Despite the described works, very few studies have attempted to determine the direct effect of music on cancer cells [1, 7]. Therefore, the aim of this work is to determine if music is capable of inducing changes in cell viability and gene expression related to apoptosis and cell-cycle control in gastric cancer cells.

Material and Methods

Cell Culture and Treatments

The human gastric cancer cell line AGS (ATCC® CRL-1739™) was obtained from "Fundación Ciencia & Vida" (Chile), donated by Dr. Lorena Lobos-González and Dr. Luis Burzio and maintained in Ham's F-12K (Kaighn's) culture medium (Corning). The medium was supplemented with 10% FBS and 100 units/mL penicillin G and 100 units/mL streptomycin. The cells were kept in a humidified incubator at 37 °C, 5% CO_2.

Measurement of Cell Viability

Cells were seeded to densities of 5000 cells/well in 96-well plates with F12-K medium and were exposed to one of two musical genres: classical (Ludwig Van Beethoven) or death metal (Cannibal Corpse), using three compositions from each genre (Table 1) that were repeated for 12 h nonstop using an iPod Shuffle MP3 player (Apple) with over-ear headphones with external noise reduction (Panasonic RP-HX250). Control cells were left in silence. Cell viability was determined by MTS reduction assay, adding 20 μL of CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay System (Promega) to the wells and incubating for 2 h. Stock solutions of the complexes were prepared in DMSO. In all experiments, the percentage of dimethyl sulfoxide (DMSO) was maintained at 0.1%. Spectrophotometric measurements were performed using a NOVOstar 700-0130 at 490 nm to determine absorbance.

Frequency Spectra

The frequency spectra of all compositions was obtained by the software Audacity version 1.3.12 (beta).

Determination of Gene Expression

RNA Extraction and Reverse Transcription AGS cells (5×10^6) were cultured while exposed to either classical or death metal music for 12 h (Table 1) in the previously described conditions. Control group cells were left in silence with the MP3 player turned off. Following musical treatment, cellular RNA was extracted using the E.Z.NA® Total RNA Kit I (OMEGA Bio-tek) according to the manufacturer's instructions. Subsequently, 500 ng of RNA was used to synthesize cDNA by Reverse Transcription using Affinity Script II (Agilent).

Gene Expression Analysis by qPCR Expression of genes related with apoptosis and cell-cycle control was evaluated in response to the musical treatments. Two microliters of cDNA was used to measure the relative expression of the genes caspases-3, 8, and 9, p53, direct IAp binding with low pI (DIABLO), p53 upregulated modulator of apoptosis (PUMA), bcl-xL, and cyclin B1 in relation to a housekeeping gene (beta-2-microglobulin, B2M). Each qPCR reaction contained 5 μl of Brilliant SYBR® Green QPCR Master Mix and 0.1 μl of each forward and reverse primers in a final volume of 10 μl. Thermocycling conditions were as follows: denaturation at 95 °C for 10 min, followed by 35 amplification cycles at 95 °C for 10 s, and then 30 s at the annealing temperature of the primer pair (58–62 °C).

Primers are shown in Table 2.

Statistical Analysis

All data was analyzed with GraphPad Prism 6 Software and expressed as mean ± standard deviation of three measurements. Statistical comparisons were made by ANOVA, and the non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison test. p values lower than 0.05 ($p < 0.05$) were considered significant.

Results

Effect of Music on Cell Viability

The effects of classical and death metal music on cell viability in gastric cancer cells AGS were determined after 12 h of treatment. As demonstrated in Fig. 1, the death metal music induces a notable increase of cellular proliferation, which is not observed with the classical music treatment. This change in proliferation suggests a preceding change in gene expression associated with apoptosis and the control of the cell cycle.

Table 1 Compositions of classical and death metal music used as treatment in AGS cells

Death metal compositions (Cannibal Corpse)	Classical compositions (Beethoven)
Death Human Collection	Piano Sonata No.15, Op.28
Blowtorch Slaughter	Bagatelle No.25 in A minor (Für Elise)
Condemned to Agony	Piano Sonata No.14 in C♯ minor, Op.27 No.2 (Moonlight Sonata)

Table 2 Primer sequences used to quantify relative gene expression

Gene	Sequence	Amplicon size
Caspase-3	FP-5′-GAG TAG ATG GTT TGA GCC TGA G-3′ RP-5′-TGC CTC ACC ACC TTT AGA AC-3′	205 bp
p53	FP-5′-ACG ACG GTG ACA CGC TTC CCT G-3′ RP-5′-CGC TAG GAT CTG ACT GCG GCT C-3′	84 bp
PUMA	FP-5′-GAC CTC AAC GCA CAG TAC GA-3′ RP-5′-GAG ATT GTA CAG GAC CCT CCA-3′	84 bp
DIABLO	FP-5′-TAA CCC TGT GTG CGG TTC CT-3′ RP-5′-ACC AAA GAC ACT GCT CTC CTC AT-3′	88 bp
Bcl-xL	FP-5′-TCG GAT CGC AGC TTG GAT GG-3′ RP-5′-GAA GCG TTC CTG GCC CTT TC-3′	139 bp
Caspase-8	FP-5′-TGA GCC CTT GAG TTG GTC ACT T-3′ RP-5′-CAG GCT CAG GAA CTT GAG GGA-3′	122 bp
Caspase-9	FP-5′-CGA GCT GTT CAG GCC CCA TA-3′ RP-5′-CGC AGA AAC GAA GCC AGC AT-3′	174 bp
Cyclin B1	FP-5′-TCA AGG ACT TAC AAA GCA CAT GAC T-3′ RP-5′-CAG CTG TGG TAG AGT GCT GAT CTT-3′	82 bp

Frequency Spectra

The frequency spectra of all compositions in both musical genres are represented in Fig. 2. Death metal frequencies range from 0 to approximately 15 kHz with very few peaks above this range, but with a high concentration of frequencies in the range of 2–6 kHz. Meanwhile, classical compositions reach only 10 kHz, and a majority of the frequencies do not exceed 3 kHz. Moreover, classical music alternates between low and moderate frequencies while death metal maintains high frequencies.

Music Can Induce Changes in Gene Expression Associated with Apoptosis and Cell-Cycle Control

We tested the effect of music on the expression profile of genes associated with apoptosis and cell-cycle control in AGS cells by qPCR after treatment of 12 h. As seen in Fig. 2, the genes cyclin B1 and caspases-3 and 8 showed a significant augmentation in expression in response to both musical treatments. Moreover, p53 expression is notably repressed with classical music but not with death metal. The opposite effect is seen in PUMA expression, that is, a notable repression following the death metal treatment but not with classical music treatment. Interestingly, classical music also induces a slow increase in the expression of bcl-xL, a known antiapoptotic gene. We do not observe changes from either musical treatment in the expression of the closely related DIABLO or caspase-9 genes.

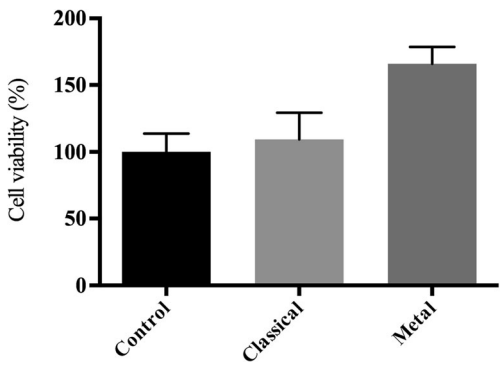

Fig. 1 Effect of classical and death metal music on cell viability in the human gastric cancer cell line AGS after 12 h of treatment. Each point is the mean ± standard deviations. Standard errors were obtained from three independent experiments

Discussion

Our novel findings show that music can induce changes in gene expression associated with apoptosis and cell-cycle control. A report from 2013 showed that music can alter the cell cycle, causing an accumulation of cells in the S phase. Moreover, this same study suggested an increase in cell death when cells were stimulated by music [1].

In our study, we noted an increase in the proliferation of AGS gastric cancer cells exposed for 12 h to death metal music, which was not observed with classical music treatment. This suggests that certain types of music are capable of altering the cell cycle in cancer cells. Almost 20 years ago, it was found that fibroblasts exposed for 10 days to treatments of 261 Hz at 87 dB for 30 s, twice daily, showed increased cell proliferation but when treatment time was augmented to 120 s, cell counts fell significantly [8]. This shows that the time of exposition to a musical stimulus can affect cellular proliferation either positively or negatively. In another study, mice were exposed for 3 days, 2 h per day, to Mozart's "Sonata for Two Pianos in D major, K. 448", then injected intraperitoneally with bromodeoxyuridine (BrdU) 24 h after the last musical exposure, and 24 h later were sacrificed to extract

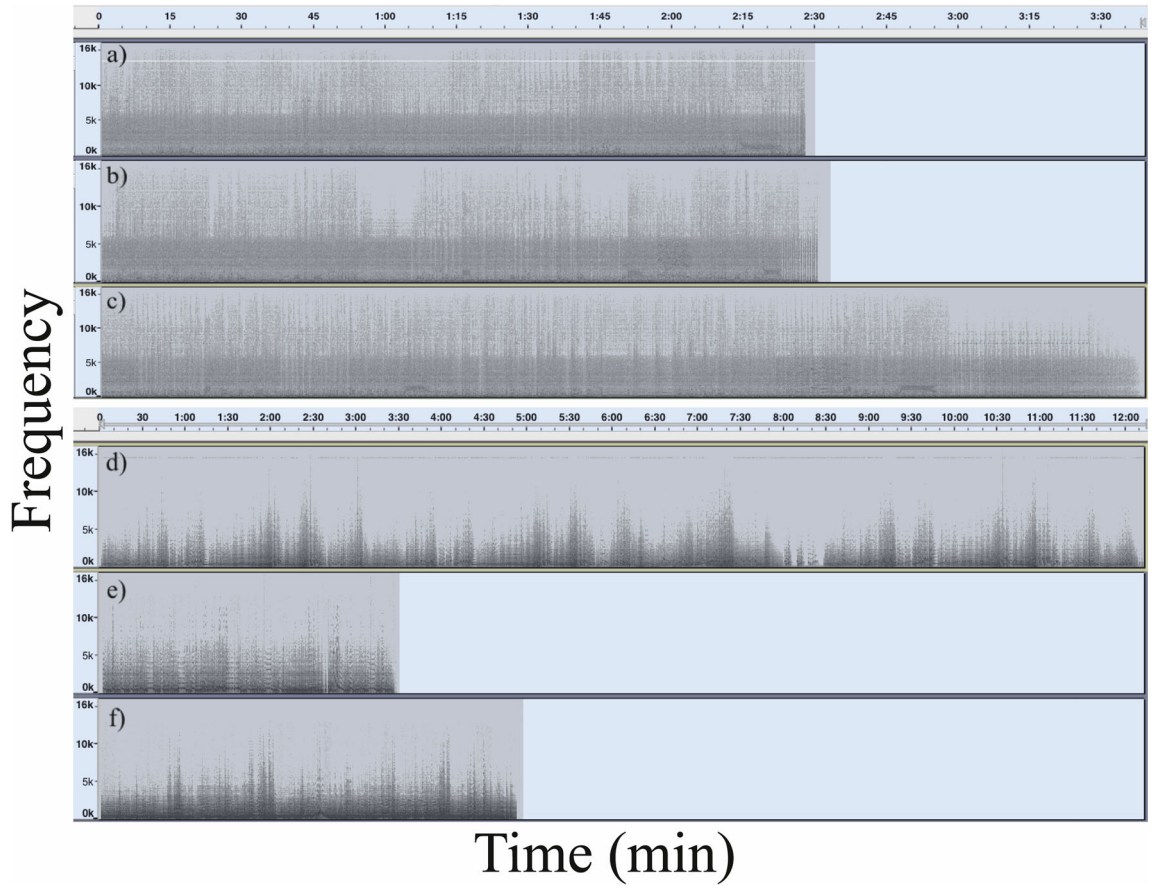

Fig. 2 Frequency spectra of the compositions used, obtained by the software Audacity 1.3.12 (beta version). Death metal compositions, **a** "Death Human Collection," **b** "Blowtorch Slaughter," and **c** "Condemned to Agony"; Classical compositions, **d** "Piano Sonata No.15, Op. 28," **e** "Bagatelle No.25 in A minor (Für Elise)," and **f** "Piano Sonata No.14 in C♯ minor, Op. 27 No.2 (Moonlight Sonata)"

their brains. Immunohistochemistry found a high number of BrdU-positive cells indicating a rise in cell proliferation due to the classical music treatment [9]. Considering our study and previous works, we believe that the different amplitude of the two musical styles could be what affects cell proliferation.

In another interesting work, breast cancer cells MCF7 were exposed for 30 min to Mozart's "Sonata for Two Pianos in D major, K. 448", Beethoven's "5th Symphony," or Ligeti's "Atmosphères," and 48 h after musical exposition, cell number and viability were measured, finding that treatment of cells with Beethoven's "5th Symphony" or Ligeti's "Atmosphères" decreased cellular viability, whereas Mozart's "Sonata" did not affect cell viability [1]. Later, this same laboratory demonstrated that Ligeti's "Atmosphères" induced early apoptosis in MCF7 and MDA-MB-231 breast cancer cells 48 h after treatment (30 min treatment time), and all three musical compositions induced late apoptosis in the cells [7].

In our study, we measured the induction of apoptosis by measuring gene expression of p53, PUMA, DIABLO, bcl-xL, cyclin B1, caspase-3, 8, and 9 in AGS gastric cancer cells exposed to classical or death metal music. We found a notable increase in expression of caspases-3 and 8 and cyclin B1 in stimulated cells, especially in the cells subjected to classical music treatment (Fig. 3). Caspases are critical protease mediators of apoptosis in different cancer cells, including gastric cancer, and are triggered by different stimuli. These stimuli include drugs such as Ganetespib [10], natural flavonoids such as galangin and quercetin [11], melittin which is the active ingredient in bee venom [12], or natural triterpenes like rosamultic acid [13]. This effect on caspase expression has never been studied as a response to music. Caspase-8 is activated by ligand-binding and activation of the death-receptors TNFR (tumor necrosis factor receptor), Fas (Fas cell surface death receptor), and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand). Once initiator caspases are activated, they signal effector caspases, like caspase-3, which is the major execution factor during apoptosis [14, 15]. This is concurrent with our observed increase in both caspase-3 and 8. A previous study found an increase in activated caspase-3 expression in cells exposed to Ligeti's "Atmosphères" [7], which was confirmed in our findings.

Regarding the genes associated with cell-cycle control, cyclin B1 is a factor that facilitates progression through the G2/M transition in the cell cycle, and its upregulation in gastric cancer is associated with less aggressive tumor behavior. Upregulation of cyclin B1 is more often observed in differentiated adenocarcinomas as opposed to in poorly differentiated adenocarcinomas

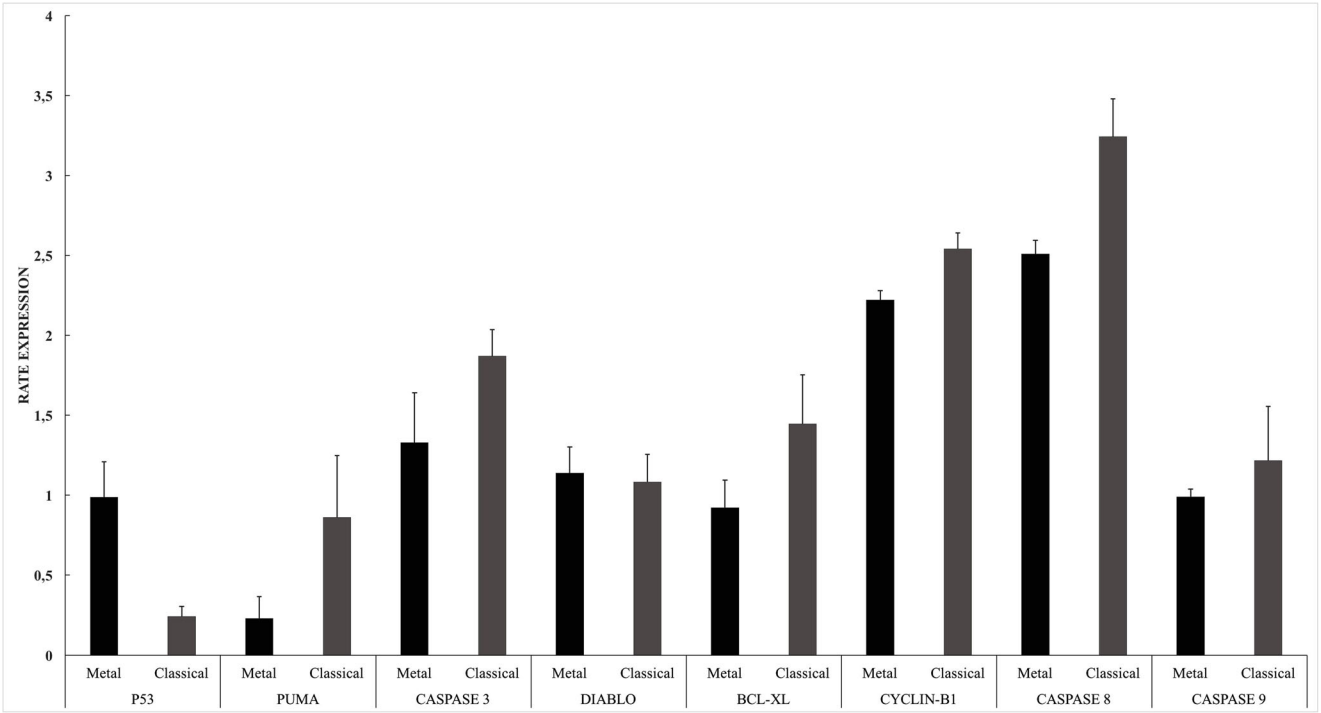

Fig. 3 Determination of gene expression in gastric cancer cells by RT-qPCR. Gene expression of p53, PUMA, caspase-3, DIABLO, bcl-xL, cyclin B1, caspase-8, and caspase-9 after gastric cancer cells AGS were exposed to 12 h of classical or death metal music. Data is presented as the mean ± SD

[16]. It is interesting that both musical stimuli can induce the overexpression of cyclin B1, which could indicate that music treatment causes cancer cells to act less aggressive. Moreover, we noted a certain expression relationship between the proapoptotic genes (caspase-3 and 8) and one gene involved in the progression of the cell cycle (cyclin B1): all three are strongly upregulated thereby suggesting that cancer cells respond to the musical stimuli by inducing apoptosis. This observation was especially notable for the classical music treatment.

Continuing the analysis of apoptotic gene expression, we observed a strong downregulation of two genes: p53 in cells exposed to classical music and PUMA in cells exposed to death metal music. PUMA is a highly efficient proapoptotic gene and p53 is one of its most studied regulators. In the apoptotic pathway, p53 is recruited to one of two p53-responsive elements in the PUMA promoter [17, 18]. In these results, the inverse relationship between PUMA and p53 expression shows that the apoptosis process does not seem to be following the same pathway in response to different musical stimuli. It is likely that both genes overexpressed early (3–6 h) in response to musical exposition, and later, expression was downregulated, as is seen at 12 h. PUMA and p53 express early on in the apoptotic pathway and are responsible for the activation and release of mitochondrial apoptogenic proteins including DIABLO and caspases [18].

Finally, we observed a small increase in the expression of the antiapoptotic gene bcl-xL in AGS cells exposed to classical music, but not in cells treated with death metal music. This difference does not seem significant.

In this work, we found for the first time that different musical genres can induce a different response considering cell proliferation and gene expression in tumor cells. More analyses are required to determine how exactly a musical stimulus can induce a response in supposedly non-auditory cancer cells, so far as to alter the expression of genes involved in apoptosis and cell-cycle control. We suspect that frequencies and amplitudes of the soundwaves can change relative gene expression, but we still do not fully understand the mechanisms behind this phenomenon. More experiments are needed to unravel this mystery because understanding the effects of a specific type of music on cancer cells could advance the potential of music therapy for cancer patients, not only for the psychological effects, but as a possible co-adjuvant to traditional treatment.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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