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# Gentamicin Induces Selective Toxicity in Metabolically Altered Vemurafenib-Resistant A375 Cells

# Metabolik Değişime Uğramış Vemurafenib Dirençli A375 Hücrelerinde Gentamisin ile Seçici Toksisitenin İndüklenmesi

**Fulya Dal Yöntem[1,2], Gökhan Ağtürk[3,4], Sinem Ayaz[3,5], Şeyma Ateşoğlu[6,7], Hülya Irmak Aksan[3], Huri Dedeakayoğulları[8], Handan Akçakaya[9], Müfide Aydoğan Ahbab[10] and Ebru Hacıosmanoğlu Aldoğan[11]**

[1]Department of Biophysics, Koç University School of Medicine, İstanbul, Türkiye.
[2]Koç University Research Center for Translational Medicine (KUTTAM), İstanbul, Türkiye.
[3]Faculty of Medicine, Haliç University, İstanbul, Türkiye.
[4]Department of Physiology, Istanbul University-Cerrahpaşa Institute of Graduate Studies, İstanbul, Türkiye.
[5]Department of Clinical Microbiology, Istanbul University-Cerrahpaşa Institute of Graduate Studies, İstanbul, Türkiye.
[6]Department of Medical Biology, Bezmialem Vakıf University Faculty of Medicine, İstanbul, Türkiye.
[7]Department of Biotechnology, Bezmialem Vakıf University Institiute of Health Sciences, İstanbul, Türkiye.
[8]Department of Medical Biochemistry, İstinye University Faculty of Medicine, İstanbul, Türkiye.
[9]Department of Biophysics, İstanbul University Faculty of Medicine, İstanbul, Türkiye.
[10]Hamidiye Vocational School of Health Services, University of Health Sciences, İstanbul, Türkiye.
[11]Department of Biophysics, Bezmialem Vakıf University Faculty of Medicine, İstanbul, Türkiye.

## ABSTRACT

Rising global cancer incidence and mortality, coupled with the challenge of drug resistance, necessitate novel therapeutic strategies. This study investigates the potential repurposing of gentamicin for treating drug-resistant melanoma by targeting metabolic alterations. Initially, we addressed the influence of antibiotics on mitochondrial function, a crucial player in oxidative phosphorylation (OXPHOS). To assess this impact, we first cultured two different cancer cells, A375 and PC3, in antibiotic-free medium and showed that mitochondrial membrane potential of cells increased in the absence of antibiotics compared to cells cultured in antibiotic containing medium. Next, we developed vemurafenib resistance in A375 cells, which were continuously cultured in antibiotic-free medium. The resistant cells exhibited a marked increase in oxygen consumption rate, indicating a shift towards OXPHOS. Finally, we treated these vemurafenib-resistant cells and noncancerous human fibroblast cells (CCD-1072Sk) with varying concentrations of gentamicin (1-1000 µM). Remarkably, gentamicin showed selective cytotoxicity towards the resistant cells while sparing non-resistant counterparts and noncancerous cells. Our findings highlight gentamicin's potential as a therapeutic agent in targeting the metabolic vulnerabilities of drug-resistant melanoma, presenting a viable new pathway in cancer treatment.

**Key Words**
Gentamicin, vemurafenib, melanoma, drug repurposing.

## ÖZ

Artan küresel kanser insidansı ve mortalitesi, ilaç direnci sorunuyla birleştiğinde yeni terapötik stratejiler gerektirmektedir. Bu çalışma, metabolik değişiklikleri hedef alarak ilaca dirençli melanoma tedavisi için gentamisinin potansiyel yeniden kullanımını araştırmaktadır. Bu çalışmada ilk olarak, oksidatif fosforilasyonda çok önemli bir oyuncu olan mitokondri fonksiyonu üzerine antibiyotiklerin etkisini ele aldık. Bu etkiyi değerlendirmek için A375 ve PC3 olmak üzere iki farklı kanser hücresini antibiyotik içermeyen ortamda çoğaltık. Antibiyotik içeren ortamda çoğaltılan hücrelere kıyasla antibiyotik içermeyen ortamda çoğaltılan hücrelerin mitokondriyal membran potansiyelinin arttığını gösterdik. Daha sonra, antibiyotik içermeyen ortamda sürekli olarak kültüre edilen A375 hücrelerinde vemurafenib direnci geliştirdik. Dirençli hücreler oksijen tüketim hızında belirgin bir artış sergileyerek oksidatif fosforilasyona doğru bir kayma olduğunu gösterdi. Son olarak, vemurafenib dirençli A375 hücrelerini ve insan fibroblast hücrelerini (CCD-1072Sk) değişen konsantrasyonlarda gentamisin (1-1000 µM) ile muamele ettik. Dikkat çekici bir şekilde gentamisin, dirençli hücrelere karşı seçici sitotoksisite gösterirken dirençli olmayan ve fibroblast hücrelerinde herhangi bir toksisite göstermemiştir. Bulgularımız, gentamisinin vemurafenib dirençli melanoma hücrelerinin metabolik zayıflıklarını hedeflemede terapötik bir ajan olarak potansiyelini vurgulamakta ve kanser tedavisinde uygulanabilir yeni bir yol sunmaktadır.

**Anahtar Kelimeler**
Gentamisin, vemurafenib, melanoma, ilaç yeniden konumlandırma.

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**Correspondence to:** F. Dal Yöntem, Department of Biophysics, Koç University, İstanbul, Türkiye.
**E-Mail:** fyontem@ku.edu.tr

## INTRODUCTION

Cancer remains one of the most serious health challenges of the 21st century. The global burden of cancer continues to rise, with estimates suggesting 18.1 million new cases and 9.6 million deaths in 2018 alone [1] However, advances in cancer therapy have yielded significant improvements in patient survival; the emergence of drug resistance is a major obstacle to lasting treatment success [2]. Despite molecular and genetics-based research aimed at cancer treatment and the development of new personalized treatments, one-third of patients diagnosed with cancer each year die [3]. Ninety percent of these deaths occur due to the tumor metastasizing to distant tissues [4]. The success of cancer treatment often remains limited due to the tumor not responding to anti-cancer drugs. This failure, appearing as drug resistance, typically results from patients' increased tolerance to pharmaceutical treatments. This concept was first identified following the observation of bacteria showing resistance to various antibiotics, and it was determined that drug resistance mechanisms exist in different diseases, including cancer [5]. Resistance to anticancer drugs not only weakens the efficacy of treatment but also contributes to the complexity and heterogeneity of tumor biology. Research has revealed that the global fight against cancer cannot be won by using drugs targeted at a single molecular pathway or genetic mutation [6]. The best example of treatments targeting a single genetic mutation and ending in failure is the BRAF V600E mutation in melanoma patients. Although treatment with the BRAF inhibitor vemurafenib, developed for melanoma patients with the V600E mutation, showed tumor shrinkage, the tumor aggressively returned within six months in the same patients [7, 8]. In addition to drug resistance developing in pioneer cancer cells and adult cancer cells, another reason for cancer recurrence in patients is explained as the existence of drug-resistant cancer cells in the heterogeneous cancer cell population even before treatment. Recent studies have revealed that some cells within heterogeneous populations possess cancer stem cell characteristics and are generally resistant to drugs [9, 10]. Therefore, cancer treatment results in the survival of drug-resistant cells, causing future pathologies while only killing drug-sensitive cells. A particular feature of drug-resistant cancer cells is the reprogramming of their energy metabolism, a phenomenon that has attracted increasing attention in the field of oncology [11]. In the 1920s, Otto Warburg demonstrated that oxygenated cancer cells consume high levels of glucose and produce high levels of lactate, showing that these cells meet their energy needs through glycolysis instead of oxidative phosphorylation [12, 13]. Known as the Warburg effect, this feature indicates that the mitochondria of cancer cells are irreversibly damaged, making energy production through glycolysis much less efficient than oxidative phosphorylation. However, recent studies have shown that mitochondria are not damaged in many different cancer types, such as leukemia, lymphoma, pancreatic ductal adenocarcinoma, melanoma, and endometrial carcinoma, and the oxidative phosphorylation process is suppressed by active glycolysis [14, 15]. Cancer cells with altered metabolism after the development of drug resistance exhibit increased diversity and adaptability, which supports their growth and progression. Consequently, the quest to overcome drug resistance has pivoted towards understanding and targeting these metabolic shifts. Recent insights into the metabolic reprogramming of drug-resistant cancer cells have triggered the exploration of inhibitors that specifically target oxidative phosphorylation [16, 17]. While the development of new drugs with such specificities is promising, it is beset with challenges, including high costs and extensive timeframes necessary for bringing new therapeutics to the market. This has directed researchers towards the strategy of drug repositioning, wherein existing drugs are repurposed for new therapeutic applications [18, 19]. In the field of drug repositioning, antibiotics have emerged as a class of compounds with potential anticancer properties. Despite the widespread clinical use of antibiotics over the past 70 years, recent studies have found that even low doses of antibiotics can inhibit mitochondrial functions and cause changes at the molecular and physiological levels [20]. In a study conducted by Lamb et al., antibiotic groups targeting the bacterial ribosomal subunit (30S), which shows structural similarity to the mitochondrial ribosomal subunit (28S), such as tetracycline and glycylcycline, were reported to slow down cancer cell growth [21]. This is exemplified by gentamicin, an aminoglycoside antibiotic that has been preliminarily associated with anticancer activities [22]. However, the potential of gentamicin as an anti-cancer agent, particularly in the context of drug-resistant cancer cells, remains underexplored.

The present study aims to bridge this gap by examining the effects of gentamicin on metabolically altered melanoma cells (A375) that have developed resistance to vemurafenib. Initially, we used two different origi-

nated cancer cells, both showing the same shift from glycolysis to oxidative phosphorylation after developing resistance to drug treatments (vemurafenib and docetaxel respectively), A375 (melanoma) and PC3 (prostate cancer) cells [16, 17]. We cultured them both in an antibiotic-free medium to study their mitochondrial function gain by analyzing membrane potential differences compared with cells grown in the presence of antibiotics to show the effect of antibiotics on mitochondrial function is not related to cell type. The subsequent step involved inducing vemurafenib resistance in the A375 cell line, followed by assessing changes in their oxygen consumption rates, which are reflective of metabolic reprogramming. Finally, we explored the effects of gentamicin across a spectrum of concentrations on both A375 and the vemurafenib-resistant A375 cells and non-cancerous human fibroblast cells (CCD-1072Sk).

## MATERIALS and METHODS

### Cell Culture and Generation of Vemurafenib Resistant A375-VR Cell Line

CCD1072-Sk (human fibroblast cells), A375 (human melanoma cells) and PC3 (human prostate cancer cells) cells were purchased from American Type Culture Collection (ATCC) and grown in DMEM (Dulbecco's Modified Eagle's Medium) for CCD1072-Sk and A375 and RPMI for PC3 cells containing 10% (v/v) fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a 5% CO2 incubator. For antibiotic free cell line generation A375 and PC3 cells were cultured in an antibiotic free medium for 5 weeks. To obtain vemurafenib resistant A375 cells, under standard culture conditions in antibiotic-free medium, cells were cultured in culture flasks containing vemurafenib (1.6 µM final concentration) in an incubator with 5% CO2 at 37°C [23]. Following the acquisition of resistance to the vemurafenib, the cells were continuously cultured in a medium containing 1 µM of vemurafenib to preserve their resistant state.

### Oxygen Consumption Rate (OCR) Analysis

OCR of A375-vemurafenib resistant (A375-VR), and non-resistant A375 cells were determined using Oxygen Consumption Rate Assay kit according to the manufacturer's protocol (Cayman, Michigan, USA). Briefly, cells were seeded in a dark 96-well plate with 6 x $10^4$ cells in each well and three replicates for each sample in 200 µl medium and kept in the incubator overnight. The next day medium in the wells was replaced with 150 µl fresh medium before analysis. The device was adjusted to 37°C before starting the experiment. Wavelengths of 380 nm for excitation and 650 nm for emission were set via the programme and the 96-well plate containing the cells was placed in the device and kinetic measurements were taken for 120 min. Fluorescence signals were converted into oxygen concentration profiles using the following transformation derived from the quenching of the phosphorescent probe by molecular oxygen:

$$[O_2](t) = \frac{[O_2]_a \, x \, I_a \, x \, (I_0 - I(t))}{I(t) x \, (I_0 - I_a)}$$

where [O2](t) is the oxygen concentration at time, [O2] a is the oxygen concentration in air-saturated buffer (oxygen contents in the starting medium were normalized assuming an O2 concentration of 235 µM at 30°C), I(t) is the fluorescent signal of the probe at time t, Ia is the signal in air-saturated buffer (baseline signal without enzyme), and I0 is the signal in deoxygenated buffer (maximal signal) [24]. The signals were corrected by subtracting the baseline fluorescence recorded in blank wells.

### Cell Viability Assay

Cell viability was determined using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (98%, Alfa Aesar) reagent. Briefly cells were seeded into 96 well plates at a density of 1 x $10^4$ cells/well and were treated with various concentrations of vemurafenib (0-100 µM) or gentamicin (0-1000 µM) for 24, 48 and 72 hours. After exposure, cells were incubated with 0.1 mg/ml MTT reagent for 2 hours. Cell culture medium was removed and 100 µL DMSO was added onto each well as stopping solution. Absorbance was measured at 570 nm using an automated microplate reader (Thermo Scientific, Multiskan GO).

### Mitochondrial Membrane Potential (ΔΨm) Measurements

A375 and PC3 cells were cultured in their respective media (DMEM for A375 and RPMI for PC3) and maintained for a period of five weeks, with and without 1% penicillin/streptomycin (P/S) supplementation. A375 and PC3 cells cultured with or without antibiotics were labeled with Rhodamine 123 (Rho123, Thermo Scientific) and the mitochondrial membrane potential (ΔΨm) was measured by flow cytometry (BD Accuri C6, Becton Dickinson). Briefly, cells were incubated with 1 µg/mL

Rhodamine 123 at 37 $^{o}$C 5% CO2 for 15 min. After incubation, cells were washed with cold PBS twice. 500 µl cold PBS were added onto cells. A minimum of 20.000 gated events were collected on flow cytometer for each sample and membrane potentials were analyzed compared to control cells.

### Statistical Analysis

All the statistical analyzes were performed using GraphPad Prism 8 (GraphPad Software Inc.; San Diego, CA, USA). All experimental data are shown as the standard deviation (SD). Significant differences were determined by using multiple t-test followed by the Holm-Sidak test for corrections. The level of $p<0.05$ was considered to indicate a statistically significant difference.

## RESULTS

### Culture medium supplemented with penicillin/streptomycin alters mitochondrial membrane potential of cells

First, we explored the impact of antibiotic presence in the culture medium on the ΔΨm of A375 cells, with PC3 prostate cancer cells included to illustrate the effect across different cell types both exerting same characteristics after developing resistance to drug treatments, a metabolic shift. The results indicated a significant increase in ΔΨm in the cells cultured without antibiotics. In the case of PC3 cells, the mean fluorescence intensity (MFI) of cells were increased from 1.019 x $10^6$ in the antibiotic-containing medium to 1.440 x $10^6$ in the antibiotic-free medium, with a 1.41-fold increase. Similarly, A375 cells showed a more pronounced increase in MFI, from 0.550 x $10^6$ in the presence of antibiotics to 0.783 x $10^6$ when cultured in the absence of antibiotics, marking a 1.42-fold increase (Figure 1).

### Vemurafenib resistance induces oxygen consumption rate at A375-VR cells

Next we developed a vemurafenib-resistant variant of the A375 cell line, A375-VR by exposing cells to gradually increasing vemurafenib concentrations, up to 1.6 µM. The establishment of the resistance was confirmed using MTT assay, which assessed cell viability after 24 and 48 hours of vemurafenib exposure across a range of concentrations (0 - 100 µM). After 24 hours, A375 cells (parental line) displayed a dose-dependent decrease in viability, with a mean viability reduction to 26.2% at the highest concentration (100 µM) (Figure 2-A). In contrast, the A375-VR cells maintained a significantly higher mean viability of 32.8% at 100 µM. After 48 hours of treatment, the differential response was even more pronounced; the A375 cells continued to exhibit decreased viability, reaching a mean of 42.9% at 100 µM. Remarkably, the A375-VR cells sustained viability but, in some cases, such as at 50 µM concentration, they even

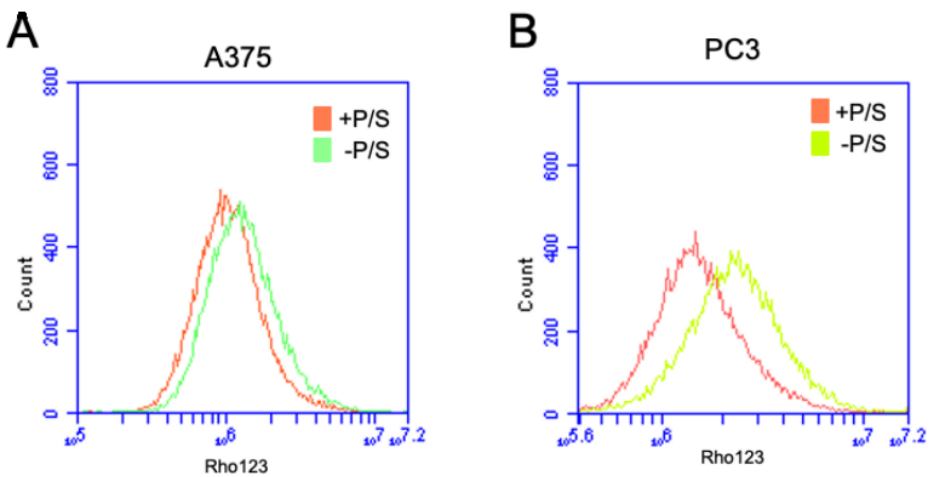


**Figure 1.** Impact of Antibiotic Supplementation on Mitochondrial Membrane Potential (ΔΨm) of A375 and PC3 Cell Lines. A) ΔΨm of A375 cells cultivated in the presence of penicillin/streptomycin (+P/S) (red) and without penicillin/streptomycin (-P/S) (green). B) ΔΨm of PC3 cells cultivated in the presence of penicillin/streptomycin (+P/S) (red) and without penicillin/streptomycin (-P/S) (green). ΔΨm were determined by flow cytometric analyses using Rhodamine 123 staining.

showed an increased mean viability of 93.8%, indicating the development of the resistance. These results confirm the successful induction of vemurafenib resistance in A375-VR cells (Figure 2).

In the evaluation of metabolic adaptations associated with vemurafenib resistance, the OCR were measured in A375 and A375-VR cell lines and we observed a dynamic shift in metabolic activity over time. Initially, the A375 cells exhibited a markedly higher OCR, nearly 4.78 times greater than the A375- VR cells. However, as the experiment progressed, the OCR of the A375-VR cells increased, surpassing that of the control cells from the 60-minute time point onwards (Figure 3).

**Gentamicin reduces cell viability of A375-VR cells**

In assessing the susceptibility of A375 and A375-VR cells to gentamicin, MTT assays were conducted after 24-hour and 48-hour treatment periods. At 24 hours, A375 cells treated with 0.1 µM gentamicin showed a viability of 82.86%, while A375-VR cells demonstrated a slightly lower viability of 77.54%. At 1 µM gentamicin concentration, the viability for A375 was 92.60%, compared to 88.94% for A375-VR. At the 10 µM concentration, A375 cell viability decreased to 59.74% and A375-VR cells showed a more pronounced decrease to 23.47%. The most significant difference was observed at 1000 µM, where A375 cells maintained a 78.02% viability, significantly higher than the A375-VR cells at 38.00% (p = 0.0085) (Figure 4-A). After 48 hours, a similar pattern was ob-

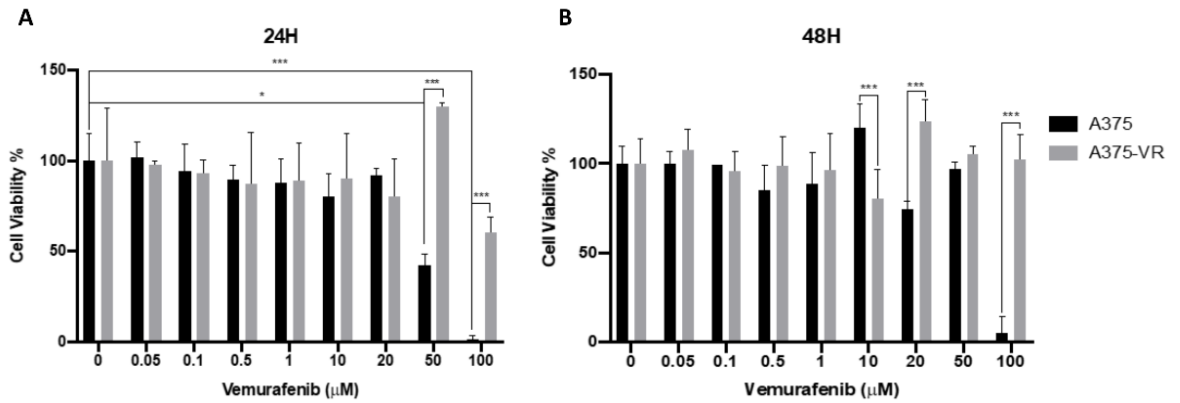


**Figure 2.** Cell Viability of A375 and Vemurafenib-Resistant A375-VR Cells upon Vemurafenib Treatment. MTT analysis of A) 24 hours and B) 48 hours A375 and A375-VR cells treated with various concentrations of vemurafenib (0.05-100 µM). *p < 0.05 and ***p < 0.001; compared with untreated control cells or compared with A375 to A375-VR at the same concentration according to multiple t-test.

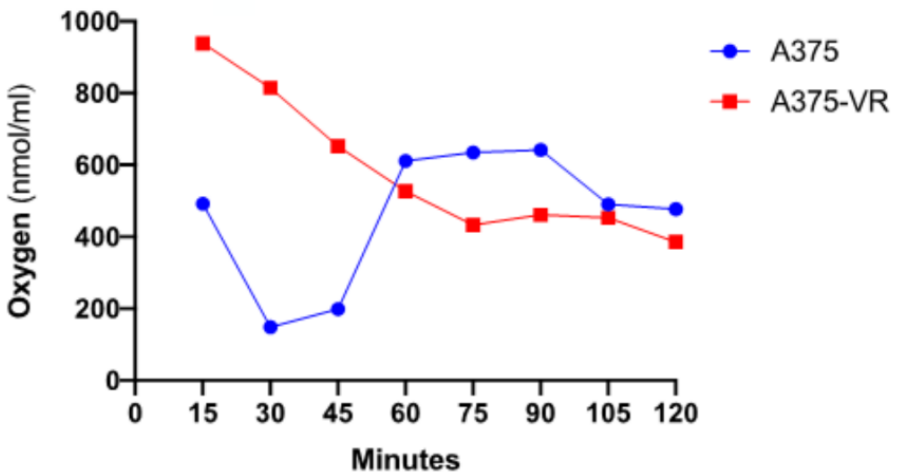


**Figure 3.** Oxygen Consumption Rates (OCR) of A375 and A375-VR Cells. OCR of A375 (blue) and A375-VR (red) across various time points (0 to 120 minutes).

served; at 0.1 µM, A375 cells had a viability of 83.66%, and A375-VR cells were at 91.63%. At 1 µM, A375 viability was at 92.94%, whereas A375-VR cells had a viability of 88.94%. At 10 µM, the viability of A375 cells decreased to 61.62%, with A375-VR cells showing a substantial decrease to 26.93%. Again, the most notable difference was at 1000 µM, where A375 cells exhibited a viability of 79.05%, significantly greater than the 31.82% viability of A375-VR cells ($p = 0.0051$) (Figure 4-B). In contrast to the cancer cell lines, CCD1072-Sk human fibroblast cells, serving as a non-cancerous control, did not exhibit a decrease in viability across the range of gentamicin concentrations tested. After 24 hours, there was no statistically significant toxicity observed at higher concentrations, with cell viability at 1000 µM and 500 µM remaining at 93.68% and 116.06%, respectively. At 48 hours, viability was maintained with a statistically significant increase at concentrations of 0.5 µM to 121% ($p = 0.0485$) and a significant decrease at 1000 µM to 88% ($p = 0.0257$), but still indicating a lack of pronounced toxicity (Figure 4-C).

## DISCUSSION

This study highlights the impact of commonly used antibiotics in cell culture environments on mitochondrial function and reveals the efficacy of gentamicin in targeting metabolic anomalies in melanoma cells, particularly those resistant to conventional treatments like vemurafenib. Our findings showed that gentamicin displayed selective cytotoxicity towards these vemurafenib-resistant cells, sparing non-resistant and non-cancerous cells, thus highlighting its potential as a therapeutic agent in targeting drug-resistant melanoma. In addition to the selective cytotoxicity of gentamicin on vemurafenib-resistant melanoma cells, we observed that A375 melanoma cells and PC3 prostate cancer cells cultured without antibiotics like penicillin-streptomycin

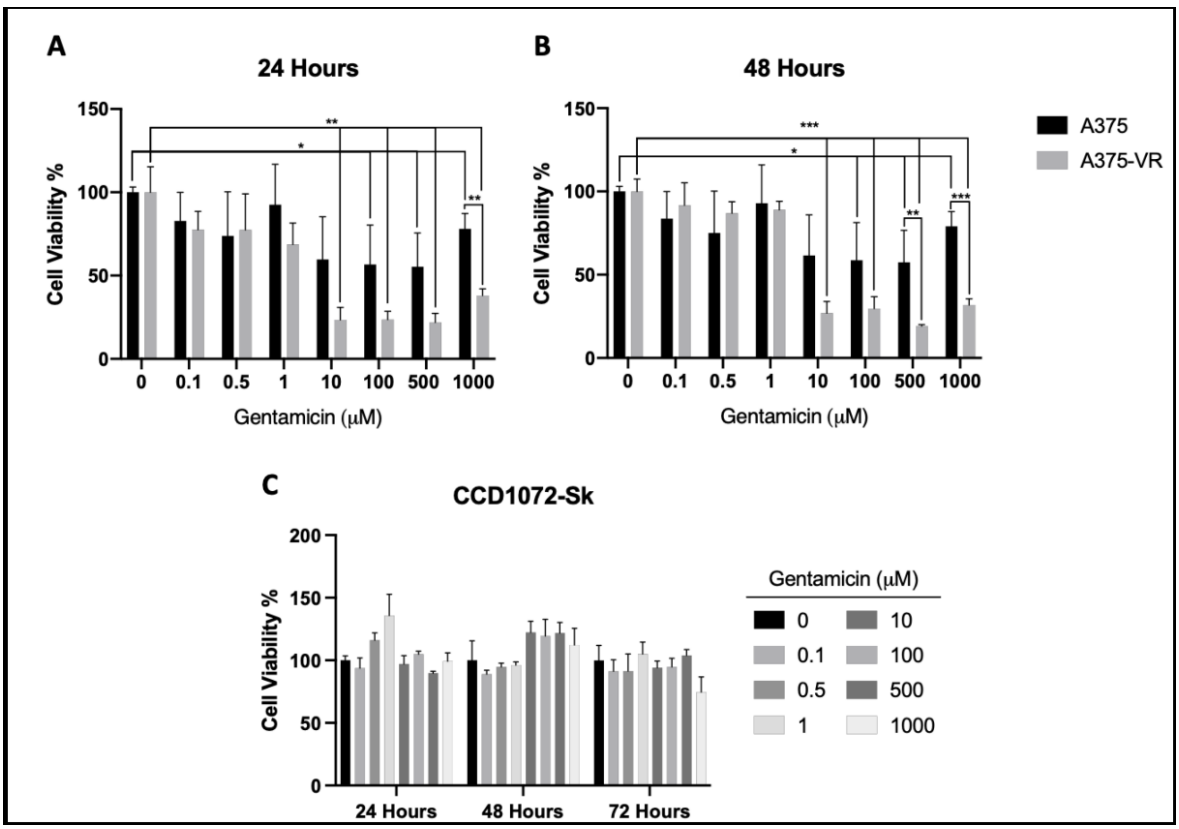


**Figure 4.** Cell Viability of A375, A375-VR, and CCD1072-Sk Cells treated with Gentamicin. MTT analysis of A375 and A375-VR cells treated with various concentrations of gentamicin (0.1-1000 µM) A) for 24 hours and B) for 48 hours. C) CCD1072-Sk cells treated with various concentrations of gentamicin (0.1-1000 µM) for 24, 48 and 72 hours. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$; compared with untreated control cells or compared with A375 to A375-VR at the same concentration according to multiple t-test.

exhibited an increase in mitochondrial membrane potential. This research contributes to understanding the complex interactions between antibiotics and cancer cell metabolism, paving the way for novel treatment strategies in melanoma therapy.

The interconnection between antibiotic action and mitochondrial functionality is not only a focal point in microbial pharmacology but also an emerging frontier in cancer research. The mitochondrion, with its endosymbiotic bacterial ancestry, shares a significant degree of homology with its prokaryotic progenitors, particularly in the structure of ribosomal subunits. This shared evolutionary legacy becomes pertinent when considering the off-target effects of antibiotics—compounds classically defined by their antimicrobial capabilities—on the bioenergetics of eukaryotic cells. Antibiotics, traditionally categorized by their antimicrobial actions, have been shown to influence mitochondrial biogenesis, dynamics, and function, which can have profound implications for cellular metabolism and viability [15, 25]. While these compounds are typically used to prevent bacterial contamination in cell culture environment, evidence suggests that they may also elicit unintended effects on mitochondrial health and activity in eukaryotic cells and animal model studies. Research by Duewelhenke et al. indicated that certain antibiotics, including quinolones, aminoglycosides, and β-lactams, can lead to mitochondrial dysfunction and increased production of reactive oxygen species (ROS) in mammalian cells. This process may result in the accumulation of oxidative damage in tissues [26]. Another study revealed that tetracyclines (such as doxycycline) that are used for gene expression-controlled models in cell culture also interfere with mitochondrial functions. They demonstrated that even at minimal concentrations, tetracyclines can trigger mitochondrial proteotoxic stress. This leads to alterations in the expression of nuclear genes, impacting mitochondrial dynamics and functionality across various organisms, including cell models, worms, flies, mice, and plants [27]. Our research contributes to the understanding of how commonly used antibiotics in cell culture, specifically penicillin-streptomycin, influence mitochondrial function by altering mitochondrial membrane potential of melanoma and prostate cancer cells. These parallel findings underscore the importance of considering the effects of standard antibiotics on cellular functions beyond their antimicrobial properties, especially in the context of cancer research and treatment.

The classical view of cancer metabolism suggests that cancer cells are predominantly glycolytic, with dysfunctional mitochondria [28]. However, recent studies revealed that many cancer cells possess functionally competent mitochondria and are capable of OXPHOS, particularly under hypoxic conditions or when confronted with metabolic stressors [29, 30]. Farge et al. found that melanoma cells exhibit a remarkable capacity to switch between metabolic pathways in response to changes in nutrient availability and the demands of rapid proliferation [31]. Similarly, Viale et al. demonstrated that pancreatic cancer cells rely on OXPHOS for survival and proliferation, especially under nutrient-depleted conditions [29]. Cancer cells that develop resistance to chemotherapy and targeted agents often undergo a metabolic shift toward OXPHOS, leveraging the mitochondria's role in biosynthesis and redox homeostasis [32, 33]. Furthermore, the metabolic reprogramming of resistant cancer cells has been implicated in the acquisition of a more aggressive and invasive phenotype. In our study similarly with the literature results we found a metabolic shift towards OXPHOS in vemurafenib-resistant A375 cells.

Gentamicin, traditionally recognized for its role as an aminoglycoside antibiotic, has a potential to disrupt mitochondrial function in cancer cells due to the structural parallels between the bacterial 30S ribosomal subunit, the primary target of gentamicin, and the mitochondrial ribosomes found in eukaryotic cells. A study by Fiorillo et al. demonstrated that aminoglycosides, including gentamicin, can induce a reduction in mitochondrial respiration and ATP production in breast cancer cells [34]. This metabolic interference, as elucidated by their work, could potentially lead to reduced cell proliferation and increased susceptibility to cell death. In addition to aminoglycosides, other classes of antibiotics have also shown promise in targeting cancer cell metabolism. A study by Wheaton et al. reported that the antibiotic chloramphenicol, which inhibits bacterial protein synthesis, can similarly target mitochondrial protein synthesis in cancer cells. Their research demonstrated that chloramphenicol could selectively kill lung cancer cells under glucose-restricted conditions, exploiting the cells' increased reliance on mitochondrial function [35]. Further supporting this approach, a study by Onoda et al. found that doxycycline can selectively target the mitochondria of human colon cancer cells. They reported that doxycycline treatment led to a significant reduction in cell viability, and induced mitochondria-medi-

ated apoptosis through both caspase-dependent and -independent pathway [36]. This notion is supported by the differential cytotoxicity observed in our study, where gentamicin exerted a markedly greater toxic effect on A375-VR cells compared to non-resistant A375 cells under the same treatment conditions. In contrast to the cancer cell lines, CCD1072-Sk human fibroblast cells, serving as a non-cancerous control, did not exhibit a decrease in viability across the range of gentamicin concentrations tested. This lack of toxicity in a non-cancerous cellular context highlights an essential therapeutic window where cancer cells, due to their altered metabolic state, can be targeted while sparing healthy cells.

The current trajectory of cancer research indicates that targeting the metabolic adaptability of cancer cells, especially those resistant to existing therapies, could indicate a new study field in oncological therapeutics. Our study not only supports the concept of metabolic reprogramming in drug-resistant cancer cells but also positions gentamicin as a potential therapeutic in this paradigm shift. Future research should aim to focus on the mechanism of gentamicin's selective toxicity, its effects on mitochondrial function, and investigate the combinatorial potential of gentamicin with other anti-cancer agents to increase its therapeutic efficacy and overcome resistance.

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