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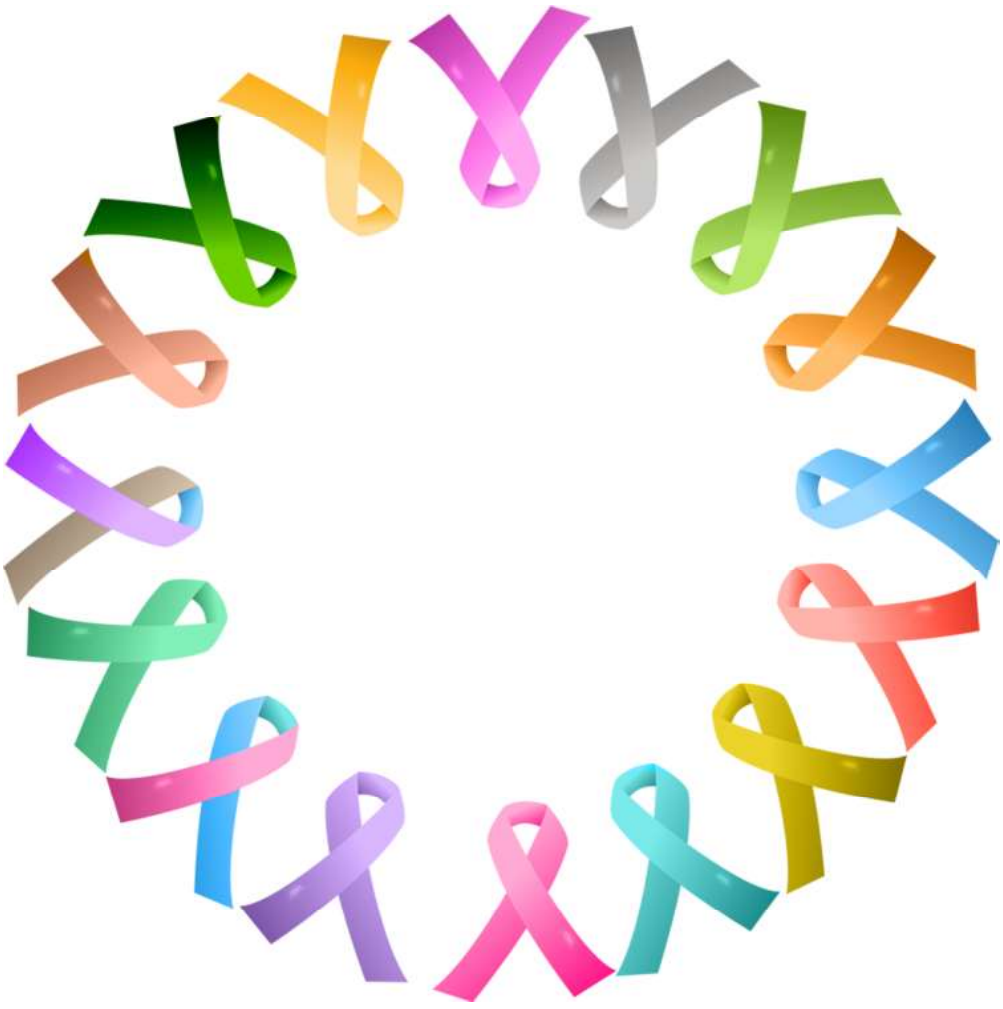

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Multidrug resistant non-small cell lung cancer cells present collateral sensitivity to platinum-based drugs

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Non-small cell lung cancer (NSCLC) is one of the most frequent cancers worldwide, with a 5-year survival rate of 15%. Conventional chemotherapy with taxanes and platinum (or alkylating) agents remains the standard treatment option for patients with NSCLC. However, multidrug resistance (MDR) remains a major obstacle, limiting the effectiveness of available treatments. Surprisingly, some drugs have been found to exert a stronger antitumor effect on MDR cells than on their counterpart sensitive cells. This effect is known as collateral sensitivity. Our work aimed to: i) select MDR NSCLC cells and establish MDR cell lines from a parental sensitive cell line; ii) employ the selected MDR cell lines to identify collateral sensitizer drugs. First, we have successfully generated two drug resistant cell lines, A549-CDR1 and A549-CDR2, by treating sensitive A549 cells with increasing concentrations of paclitaxel. These two selected cell lines were resistant to paclitaxel for at least 31 days without drug treatment, which was verified by the Sulforhodamine B (SRB) assay. Moreover, overexpression of drug efflux pumps (verified by Western Blotting – WB) and increased drug efflux (verified with the Rhodamine-123 accumulation assay) were detected in both resistant cell lines, compared to the sensitive A549 cells. In addition, our results showed that both A549-CDR1 and A549-CDR2 cells were resistant to several other anticancer drugs including docetaxel, vinorelbine, doxorubicin, etoposide and gemcitabine (evaluated by the SRB assay), confirming their MDR profile. However, and most interestingly, both MDR cell lines were more sensitive to platinum-based drugs (cisplatin, carboplatin and cyclophosphamide) than their parental cells, indicating that these drugs caused a collateral sensitivity effect in these two MDR cell lines. Since platinum-based drugs cause DNA damage, ongoing work aims to verify whether the two MDR cell lines have increased susceptibility to DNA damage, which could explain the observed collateral sensitivity effect of these drugs in the MDR cells. Overall, we established two MDR NSCLC cell lines presenting a collateral sensitivity effect to platinum-based drugs. These cell models could be a valuable tool to better understand mechanisms underlying the collateral sensitivity phenomenon to overcome chemotherapy resistance in NSCLC.
Keywords: chemotherapy, collateral sensitivity, multidrug resistance, NSCLC, platinum agents, taxanes

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Anoikis as a novel mode of shikonin derivatives anticancer action on C6 glioma cells

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Background: Shikonins are naturally occurring naphthoquinones found in the roots of several genera of the Boraginaceae family, widely known for their antiinflammatory, antioxidant, antimicrobial, and anticancer properties. This study aimed to investigate the antitumor potential of six shikonins isolated from the roots of Onosmavisianii against highly aggressive rat glioma cell line C6 and to explore the mechanisms involved. **Material and Methods:** Cell viability was estimated by MTT and CV assays. Cell death, proliferation rate, and caspase activity were assessed using

flow cytometric analysis of annexin V-FITC/propidium iodide, CFSE, and ApoStat staining, respectively. Fluorescent microscopy of propidium iodide-stained cells was employed for the detection of nuclear morphology. To evaluate the viability of detached cells, an acidic phosphatase assay was used. The cells' property to adhere was assessed by cell adhesion assay while western blot was engaged to measure the expression of relevant proteins responsible for the observed phenomenon. **Results and Conclusions:** All examined shikonins dose-dependently decreased the viability of C6 cells, with compounds 5 and 6 being the most potent ones. Compound 5 had a more profound effect on the proliferation rate of C6 cells than compound 6, resulting in almost 70% of inhibition of cell division. Additionally, compound 5, but not compound 6 generated a significant number of early and late apoptotic cells in treated cultures as detected by flow cytometry. In collision with this, typical morphological signs of apoptotic cells were not observed, and fluorescent microscopy revealed only the presence of enlarged nuclei. This paradox was resolved by the discovery of massive detached cell presence, indicating that glioma cells underwent anoikis, a cell attachment-dependent programmed cell death, in response to treatment with both agents. Decreased ability of C6 cells to adhere to the extracellular matrix and compromised integrin signaling was further confirmed by adhesion assay and western blot, respectively. Interestingly, while compound 5 triggered caspase-mediated anoikis, compound 6 realized anoikis in a caspase-independent manner and under sustained ERK1/2 activation, indicating the deviation from standard proanoikis signaling. This study represents the first proof of shikonin derivatives' strong anticancer potential realized through the inductionof anoikis of highly proliferative and invasive malignant glioma cells.
Keywords: anoikis, integrin signaling, glioma

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Different mitochondrial response in A549 KRASG12S cells and Mcf7 KRAS wild type cells to the treatment with mitochondrial superoxide radicals triggering agent 2-(1-Benzyl-4-piperidinylamino)-4-(4-chlorophenyl)-4-oxo-N-phenylbutyramide (BPCPh)

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Background: We previously published that derivatives of aroylacrylic acid phenylamides induce mitochondrial superoxide radicals (MtSR) generation and apoptosis in cancer cells. As KRAS mutant cell lines have dysfunctional mitochondria with increased basal cellular reactive oxygen species (ROS) levels, they could respond differently to MtSR triggering agents. In this study we treated A549 cells (harboring KRASG12S mutation) and Mcf7 cells (KRAS wt) to observe and compare alterations at the mitochondrial (Mt) level upon treatment with BPCPh that acts as a powerful generator of MtSR. Both cell lines are p53 wt. **Material and Methods:** Apoptosis was determined by Annexin V/propidium iodide (PI) staining and Sub-G0/G1 analysis, MtSR were detected by MitoSOX Red, total ROS by DCFDA, Mt potential was defined with MitoTracker CMX Ros and Mitochondrial potential kit/PI staining, MitoTracker Green FM was employed for Mt mass observation. Analyses were performed on FACS Calibur cytometer and Carl Zeiss fluorescent microscope**. Results:** In both A549 and Mcf7 cells apoptosis was evident after 24 hrs of BPCPh treatment. This outcome was completely reversed by N-acetylcysteine co-incubation, implying ROS generation as responsible for cell death in both cell lines. Nevertheless, at 6 hrs of treatment with 50 μM of BPCPh, the response at Mt level was drastically different in the two cell lines. Contrary to A549 cells, MtSR production in Mcf7 cells was vigorous followed by momentous boost of cellular ROS. There were no notable changes of Mt potential in A549-treated cells compared to non-treated control, but decrease in Mt mass was seen in a modest percentage of those that underwent BPCPh treatment. On the contrary, significant Mt hyperpolarization and gain in Mt mass were recorded in Mcf7 cells. Microscopic examinations showed that BPCPh treatment led to interruption of Mt networking in both cell lines. While Mt in A549 seemed to have preserved size and integrity, they have been relocated toward the plasma membrane. In Mcf7 cells, the remaining Mt were massive and repositioned near the nuclei. **Conclusion:** A549 and Mcf7 cells displayed different strategies to overcome treatment with BPCPh. While Mcf7 cells have evidently undergone mitochondrial swelling and fission, in A549 cells mitophagy may be underlying process that should be further confirmed. Our results could contribute to better understanding of Mt plasticity in cancer cells in response to strong pro-oxidant agents.
Keywords: KRASG12S mutation, mitochondrial superoxide generation, cellular ROS, apoptosis