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Research Article

Targeting p31^{comet} to Enhance Cisplatin-induced Cytotoxicity in Oral Squamous Cell Carcinoma Cells

Ana C. Henriques [1,2], João P. N. Silva [1], Bárbara Pinto [1], Patrícia M. A. Silva [1,3,4,*] and Hassan Bousbaa [1,*]

[1] UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS-CESPU), 4585-116 Gandra, Portugal; achenriques@igc.gulbenkian.pt (ACH); a30242@alunos.cespu.pt (JPNS); barbara_fernandes_pinto@hotmail.com (BP)
[2] Cell Biology of Viral Infection Lab, Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal
[3] Associate Laboratory i4HB – Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, 4585-116 Gandra, Portugal
[4] UCIBIO – Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal

* Correspondence: patricia.silva@cespu.pt (PMAS); hassan.bousbaa@iucs.cespu.pt (HB)

Abstract: p31^{comet} plays a crucial role in silencing the spindle assembly checkpoint during mitosis. This study aimed to investigate whether p31^{comet} knockdown could enhance cisplatin-induced cytotoxicity in oral cancer cells. We assessed p31^{comet} expression in oral squamous cell carcinoma (OSCC) cells and examined the impact of p31^{comet} knockdown, cisplatin treatment, and their combination on OSCC cell viability and colony formation ability. Our findings indicated an upregulation of p31^{comet} at both mRNA and protein levels in OSCC cells compared with non-cancer cells. Knockdown of p31^{comet} amplified the inhibitory effects of cisplatin on OSCC cell viability and colony formation, particularly in cells more resistant to cisplatin. This suggests that exploring the combination of p31^{comet} inhibition and cisplatin could be a potentially promising strategy to enhance sensitivity of oral cancer cells to cisplatin.

Keywords: p31^{comet}; cisplatin; combination therapy; cancer therapy; oral squamous cell carcinoma

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Introduction

Chemotherapy has been applied to cancer treatment since the 1950s [1], remaining a gold standard strategy in some cancer types, despite all the concerns related to therapeutic resistance and side effects. Some of the most widely used drugs for cancer treatment act by targeting microtubules (e.g., paclitaxel) or causing DNA damage (e.g., cisplatin) [2,3]. Since cisplatin licensing for medical use in 1978, it remains a crucial chemotherapeutic drug for the treatment of a wide range of malignancies, including oral squamous cell carcinoma (OSCC) [4]. Indeed, head and neck cancer was recently referred to as the seventh most common cancer worldwide [5]. This cancer type encompasses a heterogeneity of malignant tumors developing in the region of the head and neck [5].

Head and neck squamous cell carcinomas (HNSCC) develop from the mucosal surfaces of the oral and sinonasal cavity, as well as larynx and pharynx, representing about 90% of head and neck malignancies [4–8]. In fact, OSCC is the most common type of oral cancer, which is the sixth cause of cancer-related deaths on a global scale [9]. The preferred treatments for early stages (I and II) of OSCC are surgery alone or radiotherapy alone, with the choice being dependent on anatomical accessibility [5,9]. For the late stages (III and IV), the main treatment is surgery – surgical resection of the oral cavity and elective neck resection, and adjuvant radiotherapy or chemoradiotherapy [5,9]. Cisplatin is a first-line chemotherapeutic agent for OSCC [9], but, due to toxicity issues, in some cases, it is replaced by carboplatin, although the latter shows, in general, less efficacy [5]. Furthermore, adjuvant systemic therapy may include not only platinum agents, but also taxanes, antifolates, and cetuximab (epidermal growth factor receptor antibody), either alone or in combination [5]. Despite all the efforts that have been made, the development

of resistance and/or cross-resistance continues to hamper the success of therapy [2,10]. One of the approaches used to circumvent the limitations associated with chemotherapy is drug combination [11]. For instance, arresting cells in mitosis followed by the use of drugs that accelerate apoptosis, either by delaying mitotic slippage or by increasing death signals, is an interesting strategy proposed by Taylor and colleagues [12]. The mitotic arrest of cancer cells can be achieved through the use of antimitotic drugs. Microtubule-targeting agents (MTAs) have been the gold standard of antimitotic drugs in cancer treatment. However, the efficacy of MTAs is still limited by drug resistance and side effects [11]. The resistance to MTAs is, in part, associated with mutations in tubulin sites that affect drug binding or GTPase activity [13]. Therefore, alternative antimitotic drugs that do not target microtubules have been intensively explored [11]. In this context, spindle assembly checkpoint (SAC) regulators, such as Spindly and p31^{comet}, have emerged as promising microtubule-independent targets [11]. Notably, Spindly inhibition dramatically sensitized cancer cells to clinically relevant doses of MTAs [14]. Moreover, the SAC silencer p31^{comet} has arisen as a new potential target to increase the mitotic duration and to potentiate the lethality of MTAs or pro-apoptotic drugs in cancer cells (Fig. 1) [15,16]. Targeting SAC silencing was also demonstrated to sensitize OSCC cells to cisplatin, through inhibition of Spindly [17]. Cisplatin main target is genomic DNA (gDNA), resulting in the formation of a variety of cisplatin-DNA adducts, and cisplatin-induced DNA damage can drive apoptosis through a set of different mechanisms [2,10]. For instance, p53 activation via ATR and ATM may induce a set of processes that promote apoptosis either through intrinsic or extrinsic pathways; and the abrogation of the G1-S phase arrest induced by the activation of the cell cycle checkpoint kinases (CHK1 and CHK2) may force a premature re-entry into the cell cycle and apoptosis through the DNA repair pathway [2]. In addition to direct-targeting, cisplatin induces the formation of reactive oxygen species (ROS), which can not only damage gDNA, but also cause mitochondrial DNA damage, and mitochondrial permeability transition, promoting the release of cytochrome C and pro-caspase 9, and the activation of apoptosis [2]. In fact, the ability to overcome cisplatin-induced cell cycle arrest and to re-enter cell cycle constitutes a relevant feature in cisplatin resistance, and the regulation of SAC and miotic exit were shown to be crucial for a cisplatin resistant state of cancer cells [18]. Forcing cisplatin-treated cancer cells to arrest in mitosis was found to increase DNA damage due to impaired DNA damage repair and this, in turn, potentiated cancer cell death [19]. Therefore, by disrupting both DNA integrity and mitotic progression, this combination strategy aims to induce a more potent cytotoxic effect on cancer cells. Thus, in this work, we evaluated the relevance of p31^{comet} as a target to potentiate the cytotoxicity of cisplatin using OSCC cell lines.

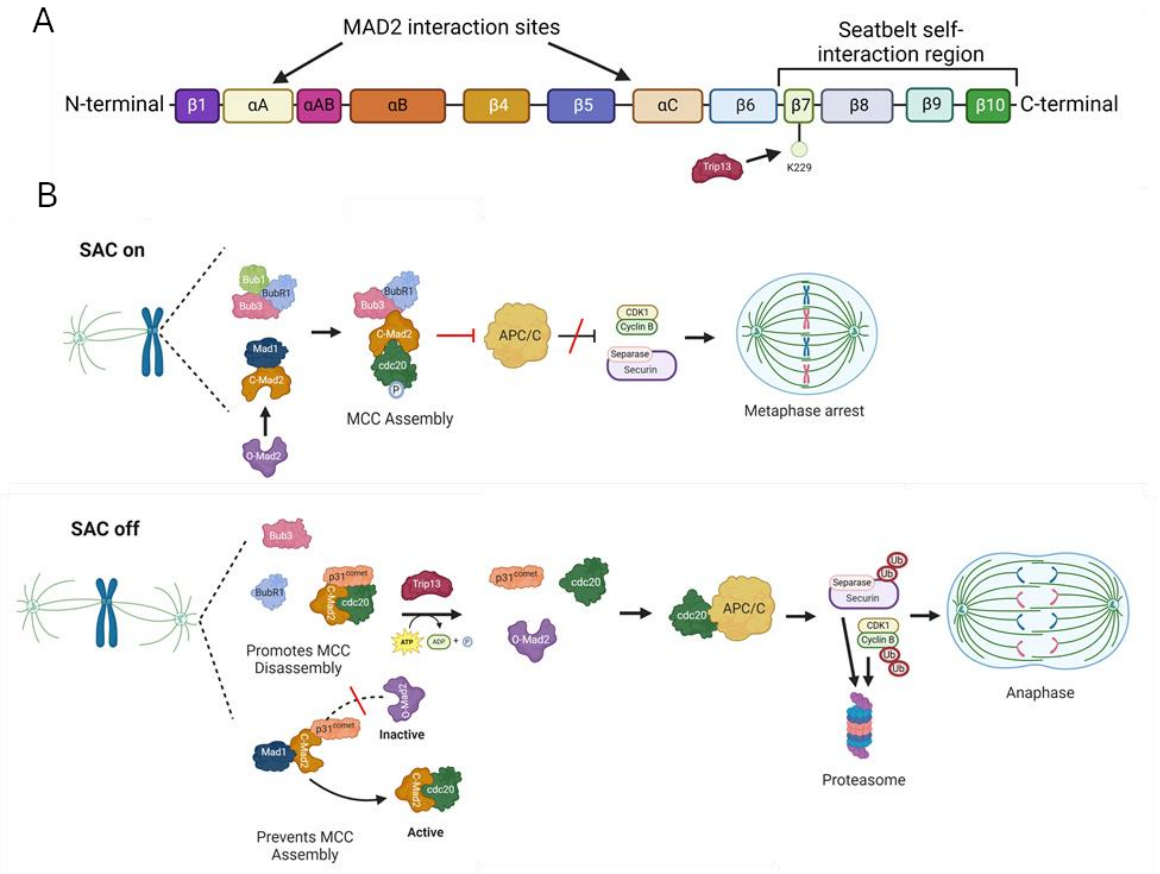

Figure 1. Schematic representation of p31^{comet} structure and its role in cell proliferation. **(A)** p31^{comet} secondary structure highlighting MAD2 and TRIP13 interaction sites. Adapted from Huang, L. *et al.* [20]. **(B)** Mechanism of Spindle Assembly Checkpoint (SAC) activation and silencing. **SAC ON:** Kinetochores unattached to microtubules lead to

the recruitment of cytosolic inactive open-MAD2 (O-MAD2) by the MAD1-closed-MAD2 (C-MAD2) template. This template can then catalyze the conversion of O-MAD2 into active C-MAD2. Then, the active form of MAD2 will interact with CDC20 and form the C-MAD2-CDC20 complex. This complex, in turn, binds to BUBR1-BUB3, leading to the assembly of the Mitotic Checkpoint Complex (MCC). The MCC inhibits the activation of the Anaphase promoting complex/cyclosome (APC/C) by preventing CDC20 activity and consequently leading to cell cycle arrest in metaphase. **SAC OFF:** When all kinetochores are correctly attached to microtubules, the SAC is silenced. The main roles of $p31^{comet}$ in SAC silencing involve both the prevention of new MCC assembly and the promotion of existing MCC disassembly. $p31^{comet}$ mediates both processes by inhibiting the conversion of O-MAD2 into C-MAD2, through binding to C-MAD2 in the MAD1-C-MAD2 complex, and by binding to C-MAD2 in the MCC complex, disrupting the interaction of BUBR1 and C-MAD2. Furthermore, $p31^{comet}$, in cooperation with TRIP13, leads to the detachment of CDC20 from C-MAD2, thus activating the APC/C, which promotes the ubiquitination of both Securin and Cyclin B, leading to cell cycle progression to anaphase. ADP: adenosine diphosphate; APC/C: anaphase promoting complex/cyclosome; ATP: adenosine triphosphate; BUB1: budding uninhibited by benzimidazoles 1; BUBR1: budding uninhibited by benzimidazole 1-related protein 1; BUB3: budding uninhibited by benzimidazole 3; CDC20: cell division cycle protein 20; CDK1: cyclin-dependent kinase 1; C-MAD2: closed mitotic arrest deficiency protein 2; K229: lysine 229; MAD1: mitotic arrest deficiency protein 1; MCC: mitotic checkpoint complex; O-Mad2: open MAD2; P: phosphate; SAC: spindle assembly checkpoint; TRIP13: thyroid hormone receptor interacting protein 13; Ub: ubiquitin.

Materials and Methods

Cell lines and culture conditions

The cell lines used in this study were grown at 37 °C, in a 5% CO_2 humidified incubator. Human oral keratinocytes (HOK) were cultured in a specific HOK medium (Innoprot, Derio, Biscaia, Spain), while OSCC cell lines SCC09 and SCC25 were cultured in DMEM-F12 culture medium (Biochrom, Holliston, MA, USA), supplemented with 10% fetal bovine serum (FBS, Biochrom) and 40 ng/ml hydrocortisone (Sigma-Aldrich, St. Louis, MO, USA).

RNA isolation and quantitative real time PCR

Total RNA isolation from culture cells and cDNA synthesis were performed as previous described [16], using the PureZOL RNA Isolation Reagent (Bio-Rad Laboratories, Hercules, CA, USA) and the iScript cDNA Synthesis Kit (Bio-Rad), according to the supplier's instructions. Amplification was performed with iQ SYBR Green Supermix Kit (Bio-Rad), on an iQ Termal Cycler (Bio-Rad) coupled to CFX Manager Sofware (version 3.1, Bio-Rad), as follows: initial denaturing step at 95.0 °C for 3 min; 40 cycles at 94.0 °C for 20 s; 65.0 °C for 30 s and 72.0 °C for 30 s. Temperatures from 65.0 to 95.0 °C, with increments of 0.5 °C every 5 s were included in the melt curves. The following primers were used: primers 5'-AGTCCCTGATTTGGAGTGGT-3' (forward) and 5'-GTAAACTGACAGCAGCCTTCC-3' (reverse) for $p31^{comet}$ [16]; 5'-AATCTGGCACCACACCTTCTA-3' (forward) and 5'-ATAGCACAGCCTGGATAGCAA-3' (reverse), for actin [14]; 5'-ACAGTCAGCCGCATCTTC-3' (forward) and 5'-GCCCAATACGACCAAATCC-3' (reverse), for GAPDH [21]. Triplicate experiments were performed, and results were analyzed through the $\Delta(\Delta CT)$ method, as previously described [16]. GAPDH and actin were used as reference controls.

siRNA transfection

Transfection was performed using the INTERFERin siRNA Transfection Reagent (PolyPlus, New York, USA), according to the manufacturer's instructions. Briefly, 24 hours after being seeded at the density of 0.12×10^6 cells/well, cells were transfected with 50 nM of a validated siRNA sequence against $p31^{comet}$ [22] or a validated negative control siRNA (AllStars Negative Control siRNA, Qiagen, Germantown MD, USA).

Cell extracts and Western blotting

Protein extraction and Western blot analysis were carried out as previously described [16]. Briefly, protein extracts were prepared with lysis buffer, in the presence of a protease inhibitor cocktail (Sigma-Aldrich), and a total of 15 µg were separated by molecular weight using a 12% SDS-PAGE gel and transferred to a nitrocellulose membrane. Rabbit anti-$p31^{comet}$ (Abcam, Cambridge, UK) and mouse anti-α-tubulin (Sigma-Aldrich) primary antibodies were diluted at 1:1,000 and 1:5,000, respectively. The horseradish peroxidase (HRP)-conjugated secondary antibodies were diluted at 1:4,000 (anti-mouse, Sigma-Aldrich) or at 1:1000 (anti-rabbit, Sigma-Aldrich). The intensity of the protein signal was quantified using ImageJ 1.4v software. α-Tubulin expression levels were used for normalization.

Cell viability assay

To determine cell viability, the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay (Sigma-Aldrich) was used. Twenty-four hours after being transfected with control or $p31^{comet}$ siRNA, cells were seeded in 96-well plates (cell density = 6,000 cells per well) and, 6 hours later, cisplatin

was added at a range of concentrations from 0 to 100 μM. The range of cisplatin concentrations was primarily selected based on previous studies conducted by our group, which are consistent with similar concentration ranges reported by others in studies with OSCC [23,24]. 24 hours later, the MTT assay was performed as previously described [16]. Optical density measurements (570 nm) were performed in a microplate reader (Biotek Synergy 2, Winooski, VT, USA), coupled to the Gen5 software (version 1.07.5, Biotek, Winooski, VT, USA). Cell viability values were normalized against control siRNA-treated cells. Three independent experiments were performed for each condition.

Colony formation assay

For the colony formation assay, 24 hours after transfection with control or p31^{comet} siRNA, cells were seeded in 6-well plates at a density of 1,000 cells per well. Six hours after seeding, cisplatin was added at the 0.25 and 0.5 μM concentrations. Lower drug doses are used in colony formation assays compared with MTT assays because cells are plated at a lower density, and the absence of cell-cell contact generally amplifies drug cytotoxic effects.Twenty-four hours later, cells were washed with PBS and allowed to grow in fresh medium for 10 days. Colonies were fixed with 3.7% (w/v) paraformaldehyde in PBS and stained with 0.05% (w/v) crystal violet (Merck Millipore, Billerica, MA, USA), as previously described [16]. Three independent experiments were performed on duplicate dishes for each condition. Plating efficiency (PE) was calculated as the percentage of the number of colonies over the number of cells seeded in the control, and the survival fraction (the number of colonies over the number of cells seeded × 1/PE) was determined.

Statistical analysis

For statistical analysis, unpaired Student t-test or ordinary two-way ANOVA with Tukey's multiple comparisons test were performed in GraphPad Prism, version 7 (GraphPad software Inc., CA, USA). Data are shown as the means ± standard deviation (SD) of at least three independent experiments.

Results

p31^{comet} expression in OSCC cell lines

Cisplatin is the first-line chemotherapeutic drug in the treatment of OSCC, but its efficacy is still limited by resistance and side effects [5]. Combination therapy has been used as a strategy to overcome such issues, and cisplatin has been administered in combination with other drugs, including antimitotics, mostly MTAs [5]. As MTAs also present limitations, in part due to the development of resistance associated with their action on microtubules, alternative antimitotic approaches have been investigated, such as the targeting of SAC regulators [5,25]. Therefore, we sought to investigate if p31^{comet} knockdown could be a valuable strategy to lower the effective dose of cisplatin in OSCC cancer and, consequently, minimize cisplatin-associated toxicity. To this end, two human OSCC cell lines, SCC09 and SCC25, were used.

p31^{comet} expression levels were first determined in both cell lines, in comparison with those of the non-cancer cell line HOK (Fig. 2). Although both OSCC lines presented higher mRNA p31^{comet} levels than the non-cancer cell line, SCC25 showed a higher p31^{comet} expression than SCC09 (Fig. 2a). These observations were consistent at protein levels (Fig. 2b). In conclusion, p31^{comet} levels were upregulated in OSCC cells, a result that was significantly more pronounced in the SCC25 cell line. p31^{comet} overexpression in OSCC cells supports the hypothesis that p31^{comet} targeting can be a potential approach to oral cancer treatment.

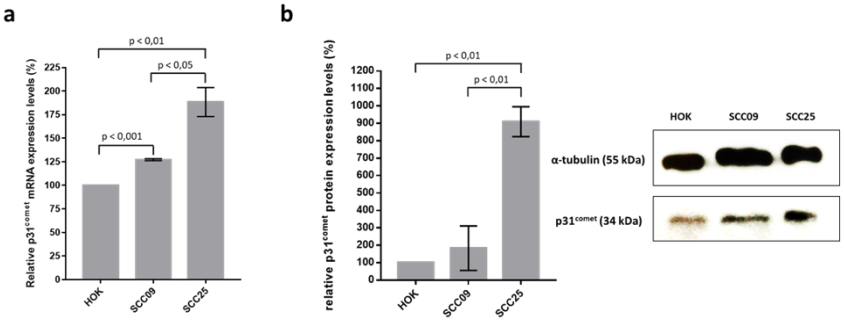

Figure 2. p31^{comet} is overexpressed in oral squamous cell carcinoma (OSCC) cells. **(a)** Relative p31^{comet} mRNA expression levels were determined by qRT-PCR in OSCC cell lines SCC09 and SCC25, comparatively to the non-cancer cell line HOK. **(b)** Representative Western blot (right) showing differential p31^{comet} protein expression levels in cells analyzed in **(a)** and the respective quantification (left). α-Tubulin was used as a loading control. Statistical analysis was performed through the Student t-test from three independent experiments. The error bars represent means ± SD.

p31^{comet} knockdown in OSCC cell lines

As p31^{comet} overexpression in OSCC cell lines reinforced the relevance of targeting p31^{comet}, siRNA-mediated p31^{comet} knockdown was then performed. Fourty-eight hours after transfection with p31^{comet} siRNA (sip31^{comet}) (Fig. 3), p31^{comet}-depletion levels were between 86 and 90% (90.38 ± 0.84 in SCC09 cells; 86.05 ± 1.58% in SCC25 cells) (Figs 3a and c) at the mRNA level, and between 52 and 56%, in both cell lines (56.45 ± 17.22% in SCC09 cells; 52.40 ± 19.74% in SCC25 cells), at the protein level (Figs 3b and d), when compared with p31^{comet} levels of cells transfected with control siRNA. As the siRNA-mediated p31^{comet} knockdown was partially achieved in OSCC cells, it was next performed in combination with cisplatin treatment.

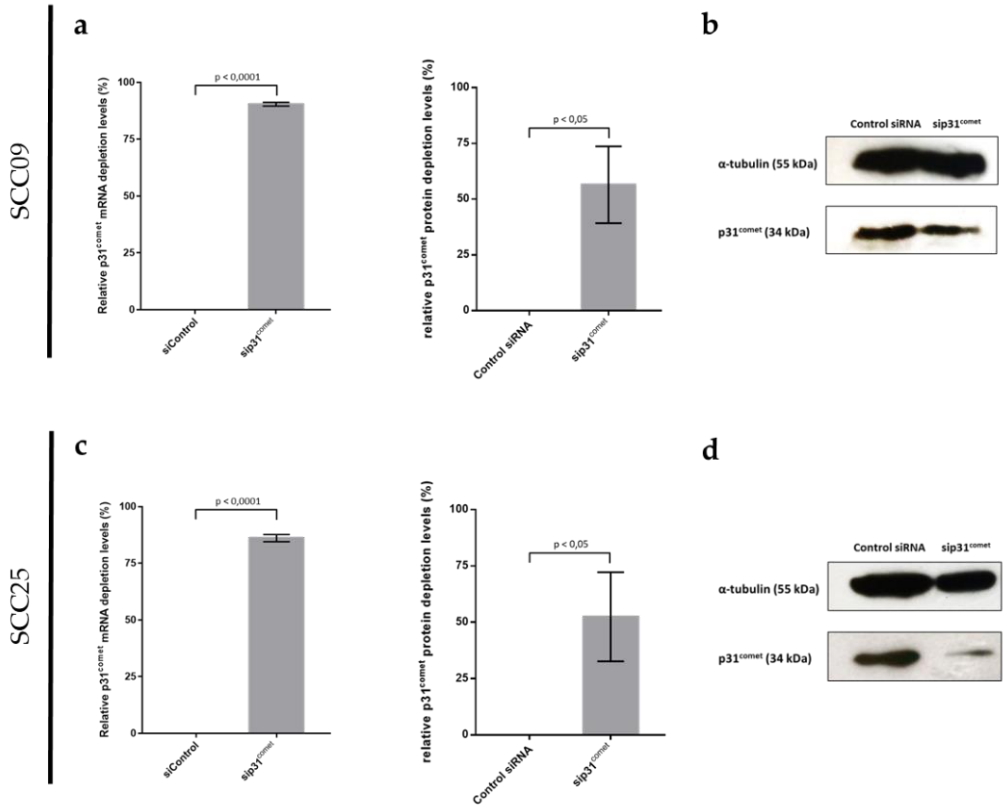

Figure 3. p31^{comet} knockdown in OSCC cells. **(a)** p31^{comet} depletion in SCC09 cells at the mRNA level, as determined by qRT-PCR, and **(b)** at the protein level, as shown by the representative Western blot (right) with the respective quantification results (left). In **(c)** and **(d)**, the same analysis as in **(a)** and **(b)**, respectively, was performed in SCC25 cells. mRNA and protein were extracted 48 h after transfection. Statistical analysis was performed through the Student t-test of three independent experiments. The error bars represent means ± SD.

p31^{comet} knockdown potentiates cisplatin-mediated toxicity in SCC09 cells

To investigate if partial p31^{comet} knockdown could potentiate the cytotoxic effect of cisplatin in the OSCC cell lines SCC09 and SCC25, MTT assays were performed. To this purpose, 24 hours after transfection with control or p31^{comet} siRNA, cells were treated with cisplatin concentrations ranging from 0 to 100 μM and incubated for another 24 hours. The results demonstrated that partial p31^{comet} knockdown significantly decreased the viability of SCC09 cells exposed to 10 μM cisplatin (a decrease of 22% or 23% relative to the individual treatment with cisplatin or sip31^{comet}, respectively; $p < 0.01$) (Fig. 4a). In agreement, the IC_{50} value of cisplatin in SCC09 cells was lower under the treatment with sip31^{comet} plus cisplatin (19.24 μM), compared with the individual treatment with cisplatin (29.12 μM) (Fig. 4c). In SCC25 cells, the combined treatment of cisplatin and sip31^{comet} did not show differences when compared with the individual treatments, at any cisplatin concentration (Fig. 4b), a fact that was also reflected in the IC_{50} values (Fig. 4d). Notably, in 10 day-colony formation assays, 0.5 μM cisplatin were enough to significantly decrease the survival of SCC09 cells in combination with sip31^{comet}, compared with individual treatments with cisplatin (a decrease of 22%, $p < 0.01$) or sip31^{comet} (a decrease of 15%, $p < 0.05$) (Fig. 4e). Regarding SCC25 cells, the combined treatment of sip31^{comet} plus 0.5 μM cisplatin decreased survival compared with the individual treatment with cisplatin (a decrease of 12%), but the difference was not statistically significant (Fig. 4f).

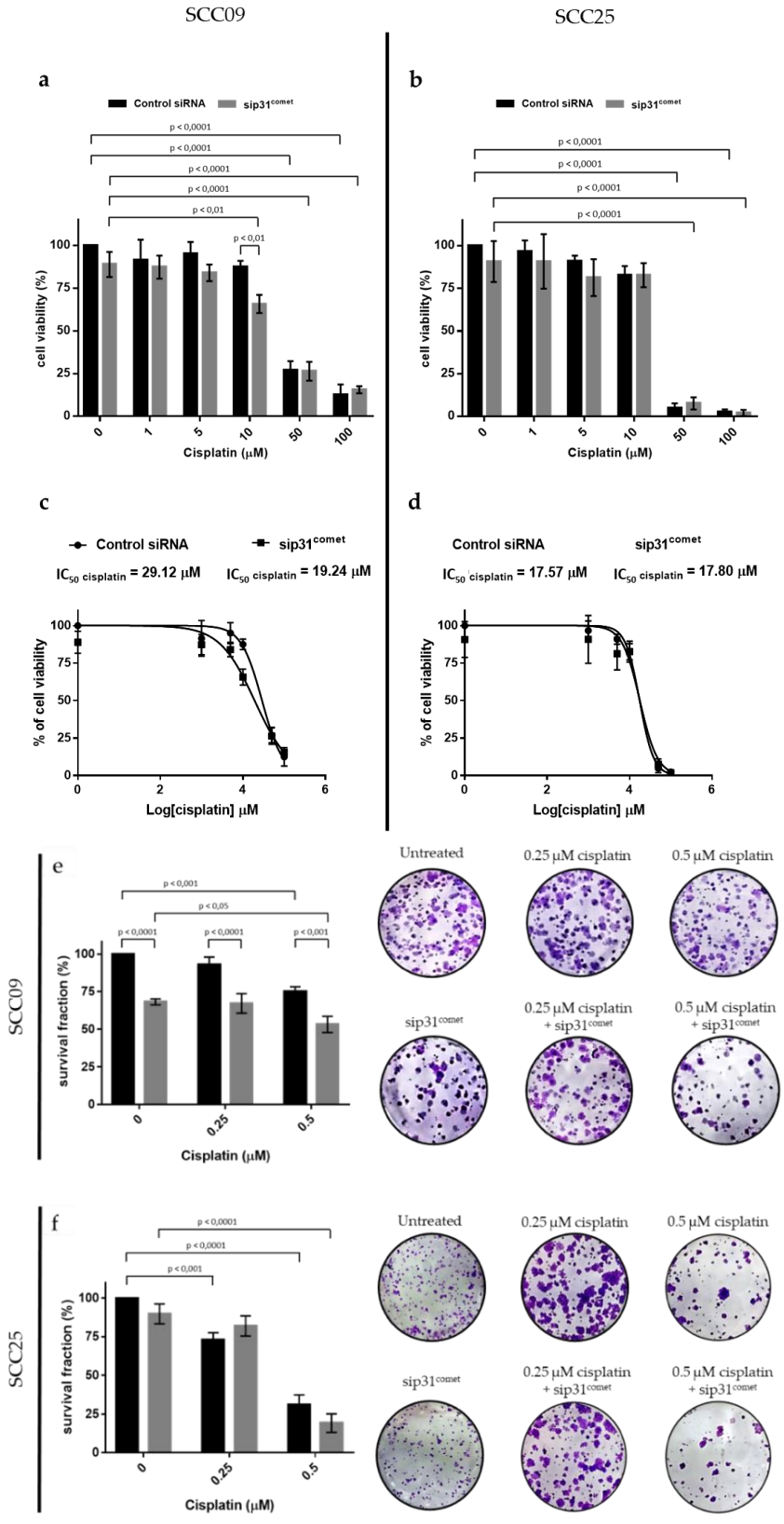

Figure 4. p31^{comet} knockdown enhances cisplatin-mediated toxicity in SCC09 cells under clinically relevant doses of cisplatin. **(a)** Cell viability of SCC09 and **(b)** SCC25 cells, as determined by the MTT assay. Cisplatin was added to SCC09 **(a)** and SCC25 **(b)** cells at the indicated concentrations (0–100 μM), 24 h after transfection with control or p31^{comet} siRNAs, and cells were incubated for an extra 24 h. **(c)** Dose response curves and IC_{50} values for cisplatin treatment of SCC09 and **(d)** SCC25 cells transfected with control or p31^{comet} siRNA. **(e)** SCC09 and **(f)** SCC25 cells were treated as in **(a)** and **(b)**, washed and allowed to grow for 10 days for colony formation assays. For each condition, representative images of surviving colonies are shown. Results are the mean from three independent experiments, expressed as the % of survival fraction. Statistical analysis was performed by a two-way ANOVA with Tukey's multiple comparisons test. The error bars represent means ± SD of three independent experiments.

The MTT assay was used to evaluate viability immediately after drug removal, and the colony formation assay to assess survival ten days after drug removal. Therefore, as in colony formation assays lower cisplatin concentrations (0.5 μM) were needed to observe the efficacy of the combined treatment (sip31^{comet} plus cisplatin) compared with individual treatments, the effects of the treatment may not be completely manifested immediately after drug removal. Interestingly, when comparing the 48h viability and the 10 day-survival values of the two cell lines treated with cisplatin only, SCC09 cells presented higher values than SCC25 cells, for the same cisplatin concentrations. This is evident, for example, at 50 μM cisplatin in MTT assays (a difference of 22% in cell viability between both cell lines), as well as for 0.5 μM of cisplatin in clonogenic assays (a difference of 44% in cell survival between both cell lines) (Fig. 5). This is also observable with the IC_{50} values under cisplatin individual treatment (29.12 μM, SCC09; 17.57 μM, SCC25). These results suggest that the SCC09 cell line is more resistant than the SCC25 cell line to cisplatin. Therefore, the results hint at the premise that targeting SAC silencing through p31^{comet} knockdown can be a valuable strategy, particularly in the OSCC cases that display resistance to cisplatin treatment.

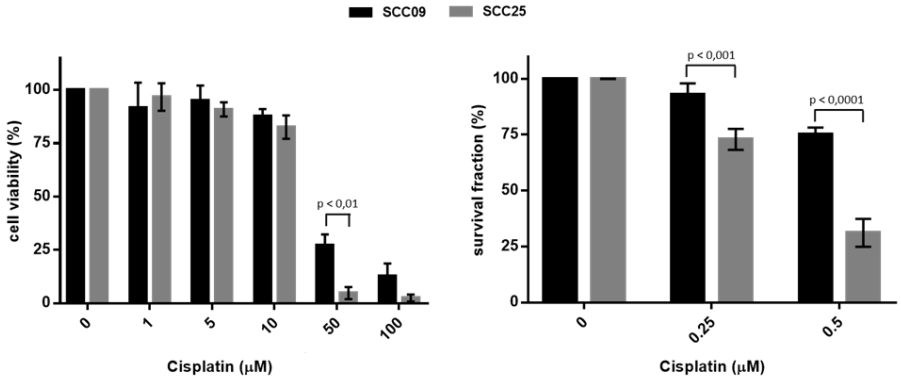

Figure 5. SCC09 cells exhibit more resistance to individual cisplatin treatment than SCC25 cells. The results of MTT and colony formation assays shown in Fig. 3 were compared between SCC09 and SCC25 cells for the individual treatment with cisplatin. Statistical analysis was performed by a two-way ANOVA with Tukey's multiple comparisons test. The error bars represent means ± SD of three independent experiments.

Discussion

Squamous carcinomas represent the majority of the head and neck cancers, and oral squamous carcinoma cells are very common among oral cancers [9]. When chemotherapy is needed, cisplatin is the first-line drug, although resistance is still a major concern [5]. To overcome this issue, combined therapy with other drugs has been explored and applied, including the combination of cisplatin with MTAs. However, the efficacy of MTAs is still hindered by the development of resistance mechanisms and side effects, and cross-resistance still occurs after the combined treatment of cisplatin and MTAs [2,10]. As the development of resistance to MTAs is, in part, related to their action on microtubules, antimitotics that do not directly interfere with microtubules have been studied [11]. For instance, targeting Spindly or BUB3 was already found to sensitize OSCC cancer cells to clinically relevant doses of cisplatin [17]. In this context, p31^{comet} also emerges as an interesting target. p31^{comet} was previously demonstrated to potentiate the lethality of the pro-apoptotic navitoclax, or the MTA paclitaxel in non-small cell lung cancer (NSCLC) cells [16]. Therefore, this study sought to exploit the relevance of the SAC silencer p31^{comet} as a possible target to potentiate the efficacy of cisplatin in OSCC cell lines. To investigate p31^{comet} significance as a possible therapeutic target, the expression of p31^{comet} was assessed at the mRNA and protein levels, being shown to be overexpressed in the OSCC cell lines analyzed at both levels. These results suggest a clinical relevance for p31^{comet} in oral cancer, either as a potential prognostic biomarker or a therapeutic target.

Indeed, this study demonstrated that p31^{comet} knockdown was able to potentiate cisplatin effect on the OSCC cell line SCC09. However, this effect was not observed in the SCC25 cell line, except for a slight decrease in cell survival in clonogenic experiments using sip31^{comet} and 0.5 μM cisplatin. This strategy would be expected to be well succeeded, as the targeting of SAC silencing through Spindly knockdown was previously shown to sensitize both SCC25 and SCC09 cell lines to cisplatin [17]. However, considering the role of p31^{comet} in mitosis, as far as known, it acts mainly at the level of SAC silencing; on the other hand, Spindly is reported to play a significant role not only in SAC silencing, but also in chromosome alignment, with its depletion being associated with severe chromosome alignment defects and extensive mitotic delay [14,26]. Therefore, we believe that the different mechanistic of p31^{comet} and Spindly could explain the differences of the results. Conversely, SCC25 displayed more sensitivity to cisplatin than SCC09 cells, suggesting that SCC09 cells are more resistant to cisplatin. Another interesting fact was that the expression of p31^{comet} was significantly higher in the SCC25 cell line (~188% at mRNA level and ~449% at the protein level) than in the SCC09 line (~127% at mRNA level and ~183% at the protein level). It is possible that the cells with lower p31^{comet} expression are more affected by its depletion, since the pool of this protein is smaller to begin with. In addition, depleting half of p31^{comet} in SCC25 cells would still mean that the SCC25 cells had more protein than SCC09 cells with no depletion. Thus, it is

possible that SCC25 cells can partially compensate for the depletion while SCC09 cells cannot. Furthermore, we believe that this finding could be related to the levels of p31^{comet} interactors in those cell lines. For instance, altered p31^{comet}/Mad2 ratios were observed in different human cancer cells, and associated with mitotic slippage, as well as with resistance to spindle poisons [15,27]. Therefore, not only p31^{comet} expression levels, but also the expression ratio to its interactors may influence the effect of p31^{comet} depletion. Thus, based on these results, it is not possible to associate the efficacy of silencing SAC through p31^{comet} targeting with basal p31^{comet} expression. Consequently, rather than p31^{comet} expression levels, other factors, including drug resistance, may dictate if p31^{comet} knockdown can be a useful strategy in the treatment of OSCC. Although our primary focus was to assess the effectiveness of p31^{comet} as a target to enhance cisplatin cytotoxicity against oral cancer cells, and we do not expect significant differences in behavior between cancer and non-cancer lines, as cisplatin targets are common to both, the inclusion of non-cancer cells could be considered in future studies examining the impact of this combination on both non-cancer cells, and other cancer types. Furthermore, evaluating p31^{comet} expression in patient samples, both with and without previous cisplatin treatment, could provide valuable insights. Such studies are crucial for comprehensively evaluating the therapeutic potential of this treatment approach. Therefore, the combination of p31^{comet} inhibition and cisplatin is a promising strategy that deserves to be explored for oral cancer in the context of cisplatin resistance, independently of p31^{comet} expression status. In conclusion, p31^{comet} knockdown potentiated cisplatin effect in the most resistant OSCC cell line. Although further studies are needed to confirm this finding, the results pave the way for an alternative antimitotic strategy to potentiate cisplatin effect in oral cancer.

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Author Contributions

HB and PMAS conceived and designed the study and supervised the work. ACH performed the experiments, collected and analyzed experimental data. ACH, JPNS, BP, PMAS and HB drafted the manuscript. All authors revised the manuscript. HB acquired and managed the funding. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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