Resistance to the platinum-based chemotherapeutic drugs in oral cancer: Focus on the role of p22phox (Review)

JIN-CHING LEE[1-4*], CHING-YING WU[5,6*], TSAI-HUI DUH[7], TAI-JAN CHIU[8,9], CHIEN-CHIH CHIU[4], CHIU-HSIEN LEE[10] and JEFF YI-FU CHEN[4]

[1]Department of Marine Biotechnology and Resources, College of Marine Sciences, National Sun Yat-sen University, Kaohsiung 804201, Taiwan, R.O.C; [2]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807378, Taiwan, R.O.C; [3]Center for Tropic Medicine and Infectious Disease Research, Kaohsiung Medical University, Kaohsiung 807378, Taiwan, R.O.C; [4]Department of Biotechnology, College of Life Science, Kaohsiung Medical University, Kaohsiung 807378, Taiwan, R.O.C; [5]College of Medicine and Post-Baccalaureate Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan, R.O.C; [6]Department of Dermatology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University Hospital, Kaohsiung 80145, Taiwan, R.O.C; [7]Department of Medicinal and Applied Chemistry, College of Life Science, Kaohsiung Medical University, Kaohsiung 807378, Taiwan, R.O.C; [8]Department of Hematology-Oncology, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University College of Medicine, Kaohsiung 833401, Taiwan, R.O.C; [9]Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan 33305, Taiwan, R.O.C; [10]National Guangfu Commercial and Industrial Vocational High School, Hualien 976001, Taiwan, R.O.C

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Abstract. Oral cancer, commonly known as oral squamous cell carcinoma (OSCC), is an aggressive malignancy in the oral cavity with a poor prognosis and survival rate, particularly at the advanced stages. Oral cancer represents one of the most widespread cancers worldwide, in which the prevalence is particularly high in South and Southeast Asia. While the incidence and mortality rates continue to increase over the past decades, oral cancer treatment can be challenging and at times ineffective, largely due to drug resistance. To date, platinum-based drugs, such as cisplatin, remain the mainstay of chemotherapy for patients with oral cancer. However, long-term exposure to cisplatin inevitably leads to the development of resistance to the drug, which is still a major issue to overcome in oral cancer treatment. The molecular mechanisms of cisplatin resistance in oral cancer have been extensively studied in recent years and the present review places specific emphasis on a novel mechanism of resistance to the platinum drugs mediated by p22phox, an endoplasmic reticulum membrane protein. In addition to delineating the unique p22phox-dependent cisplatin resistance, the present review compares and contrasts the resistance mechanism to its current counterparts. Finally, with the goal of tackling the problem of chemotherapy resistance in oral cancer, various strategies are presented that may counteract p22phox-dependent cisplatin resistance, which may potentially improve the efficacy of the platinum-based drugs and warrant future clinical validation.

Contents

1. Introduction

Oral cancer is a malignant neoplasm occurring in the oral cavity and is the sixth most common cancer globally (1), accounting for an estimated 177,757 cancer-associated deaths in 2020 (2). More than 90% of oral cancer cases belong to the histological category of oral squamous cell carcinoma (OSCC). Oral cancer is highly prevalent in certain geographic

Correspondence to: Dr Jeff Yi-Fu Chen, Department of Biotechnology, College of Life Science, Kaohsiung Medical University, 100 Shih-Chuan First Road, San-Ming, Kaohsiung 807378, Taiwan, R.O.C
E-mail: yifuc@kmu.edu.tw

*Contributed equally

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locations of the world, including South and Southeast Asia. In Taiwan, according to the annual statistics of the Ministry of Health and Welfare, oral cancer ranked sixth and fifth in terms of cancer incidence and mortality rates, respectively, in 2020 (3). Of note, oral cancer cases and deaths continue to increase in recent years (4). While alcohol consumption and cigarette smoking are significant risk factors for oral cancer, betel nut chewing in South and Southeast Asian countries is also closely associated with the disease (5). Furthermore, human papillomavirus infection has been linked to oral cancer (6). In addition, molecular alterations may contribute to the development of oral cancer. For instance, genetic mutations that lead to either activation of proto-oncogenes, such as epidermal growth factor receptor (EGFR) (7), or inactivation of tumor suppressor genes, such as p53 (8), may increase the risk of oral cancer. The average 5-year survival rate of oral cancer shows a marked decline as the disease progresses to advanced clinical stages. In Taiwan, the 5-year survival rate drops significantly from 78.98% at stage I to a dismal 36.17% at stage IV (9). Therefore, early diagnosis and treatment should substantially increase the survival rate of oral cancer.

Owing to its extraordinarily heterogeneous nature, the treatment of oral cancer has been challenging. The treatment options for oral cancer usually depend on the tumor's location and stage, as well as the patient's health conditions and preferences. Nowadays, surgery, if resectable, remains the mainstay of treatment for oral cancer, which can be followed by adjuvant radiotherapy alone or concurrent chemoradiotherapy (CCRT), particularly for unresectable locoregionally advanced tumors (10). In addition, cetuximab, an antibody-based agent that inhibits EGFR signaling, exhibits significant treatment efficacy in locally advanced and metastatic oral cancer (11). Of note, there is emerging evidence that immune checkpoint inhibitors, such as anti-programmed cell death-1 antibody, may reinvigorate T cells in the tumor microenvironment (TME) and enhance anti-tumor immunity in oral cancer (12,13). Among the treatment options for oral cancer, platinum-based drugs, such as cisplatin, carboplatin and oxaliplatin, have been commonly used in chemotherapy regimens and proven to decrease locoregional recurrence and improve disease-free survival (DFS) in patients with advanced OSCC (14). However, aside from various toxic side effects, resistance to platinum drugs remains a major obstacle to effective treatment for oral cancer. Thus, understanding the molecular basis of chemoresistance to platinum drugs is crucial for overcoming resistance and developing promising treatment strategies.

According to recent studies, chemoresistance to platinum drugs in oral cancer, including cisplatin resistance, has been attributed to tremendously diverse molecular mechanisms involving DNA damage response, epigenetic modifications, programmed cell death, TME and cellular transport (15,16). While the role of p22phox, an endoplasmic reticulum (ER) membrane protein, in mediating resistance to the platinum drugs in OSCC has been previously demonstrated by our group (17-19), the mechanism of action is different from those mentioned above. The current report focused on reviewing this unique and novel drug resistance mechanism and comparing it to the currently known mechanisms where appropriate. Furthermore, based on this p22phox-dependent chemoresistance, future perspectives of better therapeutic strategies for oral cancer were delineated.

2. p22phox and cancer

p22phox is an essential component of the membrane-bound NADPH oxidase (Nox) proteins, a family of redox enzymes that generate the majority of intracellular reactive oxygen species (ROS) (20). There are seven Nox family members in humans, namely Nox1-5 and dual oxidase 1-2. Each Nox is activated as it is translocated to the membrane, forming a complex consisting of p22phox and several cytosolic regulatory subunits. p22phox was first identified as the binding partner of Nox2 in human granulocytes (21), constituting the Nox2-p22phox heterodimer later shown to be the catalytic core of the phagocyte Nox. p22phox functions to stabilize its Nox binding partners, which is required for optimal Nox enzyme activity at the membrane. Supporting this notion are studies demonstrating that downregulation of p22phox expression by small interfering RNAs (siRNAs) and truncation of the proline-rich region of p22phox protein inhibit ROS production by several Nox enzymes (22,23). Despite being widely regarded as an ER protein, the function of p22phox in ER has not been completely elucidated. The association of Nox enzymes with p22phox in ER appears to be a prerequisite for proper localization of the heterodimers to membrane compartments of specific organelles (24,25). In addition, p22phox is a target of unfolded protein response (UPR) transcription factor ATF4 under ER stress, which mediates ER stress and promotes the UPR *in vitro* and *in vivo* (26).

It is generally thought that ROS has a key role in cancer progression and development. Excessive amounts of ROS and downregulation of ROS scavengers are crucial hallmarks of cancer. Despite being essential for the activity of the Nox enzymes, the role of p22phox in cancer has remained largely elusive. A recent report indicates that p22phox acts as a pivotal oncogene that enhances cancer cell proliferation and tumorigenesis in ovarian cancer (27). It has also been shown that p22phox can inactivate tumor suppressor protein tuberin by enhancing Akt-dependent phosphorylation of tuberin in renal cell carcinoma (28). In pancreatic cells, transcriptional upregulation of p22phox by an Akt-dependent pathway leads to increased Nox activity and inhibition of apoptosis (29). Furthermore, p22phox can promote tumor angiogenesis and growth through Akt and ERK1/2 signaling pathways in prostate cancer (30). Previously, no indication linking p22phox to oral cancer was available until studies by our group revealed the role of p22phox in chemotherapy resistance of oral cancer (17-19). However, the involvement of p22phox in chemoresistance in other types of cancer is virtually unknown, except for one study in which elevated p22phox expression is significantly associated with resistance to chemotherapy in EGFR-tyrosine kinase inhibitor (TKI)-resistant lung adenocarcinoma (31).

3. Cisplatin in oral cancer treatment

Cisplatin, or cis-diamminedichloroplatinum (II) (CDDP), is an alkylating compound that has been widely used as a platinum-based chemotherapeutic agent with potent antitumor

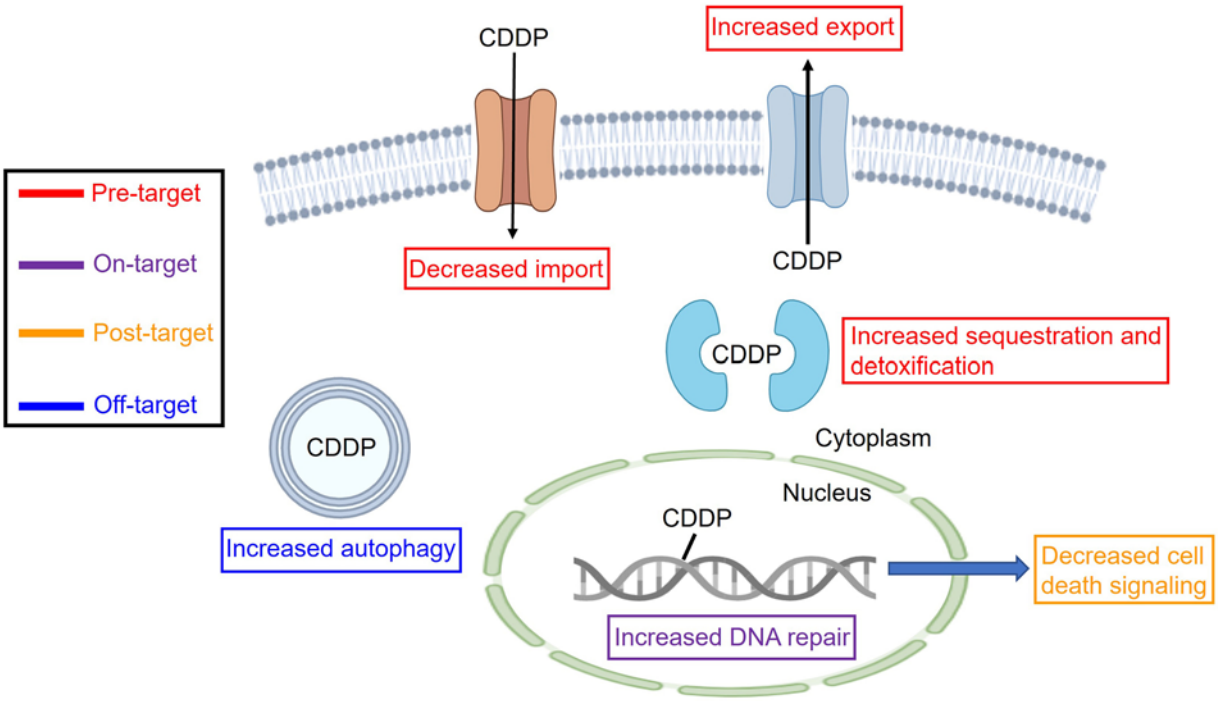

Figure 1. Current understanding of the molecular mechanisms of CDDP resistance. Based on the mode, site and hierarchy of action, the mechanisms can be classified into four categories: i) Processes preceding the binding of CDDP to its nuclear DNA target (pre-target resistance); ii) processes enabling the cell to repair DNA damage caused by CDDP (on-target resistance); iii) processes interfering with the damaged DNA-induced cell senescence or death signals (post-target resistance); iv) processes stimulating pro-survival but not CDDP-associated signals that counteract CDDP-induced cytotoxicity (off-target resistance). Created with BioRender.com. CDDP, cisplatin.

effects against various solid tumors over decades. CDDP has the ability to induce DNA crosslinks and adducts, which causes DNA damage and blocks DNA replication. In response to this DNA insult, cells may activate several DNA damage recognition proteins, including nucleotide excision repair (NER) proteins, mismatch repair proteins and high-mobility group proteins (32). The DNA damage signals are later translated into DNA repair or cell cycle arrest, culminating in the induction of programmed cell death if the repair mechanisms are overwhelmed by the damage. However, attenuation of DNA damage-induced apoptotic signals may largely contribute to CDDP resistance.

Despite the problem of drug resistance, clinically, CDDP is extensively used in chemotherapy for a variety of cancers, including testicular, ovarian, breast, lung, bladder, cervical and oral cancers (33). Particularly in oral cancer, CDDP combined with other chemotherapy drugs and radiation therapy has been the major treatment regimen. For instance, sequential combination treatment of oral cancer cells with CDDP followed by 5-fluorouracil (5-FU) increased the induction of apoptosis in the cells (34) and improved the survival of patients with advanced OSCC (35). Preoperative induction chemotherapy with CDDP and other chemotherapeutic agents, such as docetaxel and 5-FU, may yield better treatment outcomes and improve overall survival in selected patients (36). Postoperative radiotherapy concurrent with CDDP treatment significantly improved the prognosis of high-risk patients with OSCC (14). In addition, the combination of CDDP and EGFR inhibitors augmented the sensitivity of CDDP-resistant OSCC cells to CDDP (37,38). However, similar to other cancer types, long-term exposure to CDDP inevitably develops drug resistance in oral cancer. Therefore, to increase treatment efficacy, reinforcement of sensitivity to CDDP is a major issue to address.

4. Cisplatin resistance in oral cancer

Chronic exposure to CDDP may result in acquired resistance to the drug, which significantly reduces the efficacy of cancer chemotherapy. Cancer cells may become resistant to CDDP-induced cytotoxicity because of a broad range of genetic or epigenetic changes. Based on the mode of action and the hierarchical action sequence, the impacts of these alterations can be classified into the following categories: i) Influence on processes preceding CDDP-DNA binding occurring in the nucleus (pre-target resistance); ii) enhancement of DNA damage repair and tolerance elicited by CDDP (on-target resistance); iii) impairment of the cell death signaling pathways activated by CDDP-elicited DNA damage (post-target resistance); iv) stimulation of molecular circuitries of pro-survival signals that are not closely associated with, or even irrelevant to, CDDP-induced signals (off-target resistance) (39) (Fig. 1). For instance, in pre-target resistance, decreased uptake or increased efflux of CDDP across the plasma membrane through specific transporters can reduce the amount of CDDP in the cytoplasm (40,41). Furthermore, high levels of detoxification-related factors, such as glutathione (GSH), glutathione S transferase (GST) and metallothioneins (MTs), may contribute to CDDP resistance by increasing the cytoplasmic CDDP buffering capacity (42,43). In on-target resistance, an enhanced NER system may counteract CDDP-induced DNA damage, predicting poorer prognosis in patients treated

with CDDP-based CCRT (44,45). Furthermore, defects in p53 signaling or in several pro-apoptotic signal transducers such as $p38^{MAPK}$ and JNK1 may render cells less sensitive to CDDP-induced cytotoxicity, belonging to the post-target resistance (46,47). Finally, upregulation of autophagy is essential for acquired CDDP resistance in lung adenocarcinoma, representing one of the off-target mechanisms (48). However, it is worth noting that concurrent activation of numerous non-overlapping mechanisms is necessary to counteract the cytotoxic effect of CDDP at multiple levels, which may explain why efficient strategies to tackle CDDP resistance are still lacking.

In general, the molecular mechanisms of CDDP resistance in oral cancer fall into the aforementioned categories. For instance, upregulation of excision repair cross-complementation group 1 leads to CDDP resistance and is associated with poor prognosis in OSCC (49,50), representing the on-target resistance. In addition, overexpression of ATP-binding cassette (ABC) drug efflux transporters, including ABCB1, has been implicated in CDDP resistance in various solid tumors (51). In OSCC, circular (circ) non-coding RNA circ_0109291 promotes CDDP resistance by increasing ABCB1 expression, exemplifying an epigenetic mechanism of the pre-target category (52). A hypoxic TME can enhance the expression of hypoxia-inducible factor-1α and confer resistance to chemoradiotherapy in OSCC by inhibiting the pro-apoptotic but promoting the anti-apoptotic signals, an example of the post-target mechanism (53). Finally, increased autophagic flux and autophagosome formation were observed in CDDP-resistant OSCC cells, which may be classified into the off-target mechanism (54). Of note, there are still a plethora of mechanisms of resistance to CDDP in OSCC that have not been definitively categorized, including the focus of the present review, i.e., p22phox-dependent CDDP resistance.

5. p22phox confers differential resistance to platinum-based drugs

As mentioned, except for one report where p22phox expression is significantly associated with chemosensitivity in EGFR-TKI-resistant lung adenocarcinoma (31), evidence demonstrating the involvement of p22phox in chemoresistance was previously lacking until results were published by our group (17-19). The studies by our group showed that p22phox expression was significantly higher in CDDP-resistant than in CDDP-sensitive OSCC tumors, suggesting a clinical association between p22phox and CDDP resistance in patients with OSCC. OSCC cells ectopically overexpressing p22phox acquired resistance to CDDP and carboplatin, and to a much lesser extent to oxaliplatin; conversely and consistently, short hairpin RNA-mediated knockdown of p22phox sensitized the cells to the platinum drugs to different degrees. Given that oxaliplatin is the third-generation platinum drug with milder side effects than CDDP (55,56), results from our group may suggest oxaliplatin as a chemotherapeutic option, particularly for p22phox-overexpressing OSCC that is intrinsically insensitive to CDDP (19). On the other hand, it was found that p22phox overexpression also had little impact on the cytotoxic effect of 5-FU, an antimetabolite drug that interferes with DNA synthesis and has been widely used in oral cancer treatment (19,35). In conclusion, it may be feasible that 5-FU combined with oxaliplatin could deliver a more effective and safer treatment for p22phox-overexpressing OSCC.

It has been shown that all of the three platinum drugs can form DNA adducts and elicit apoptosis (57). Thus, our group investigated whether p22phox expression modulates resistance to platinum drugs through apoptotic signals. Indeed, p22phox overexpression suppressed, while p22phox knockdown promoted, caspase-dependent apoptosis in OSCC cells treated with the drugs (18,19). Furthermore, consistently, regardless of the expression level of p22phox, changes in the apoptotic signals elicited by oxaliplatin treatment were markedly less significant than those by CDDP or carboplatin treatment (19). These results indicate that p22phox expression has a lesser effect on oxaliplatin-induced cytotoxicity, thus confirming the observed differential resistance to the three platinum drugs. It is worth noting that, at the treatment doses that caused comparable cytotoxicity on the OSCC cells, oxaliplatin triggered significantly reduced caspase-dependent signals compared with CDDP and carboplatin, suggesting that oxaliplatin, in addition to forming DNA adducts and inducing apoptosis, may adopt apoptosis-independent mechanisms to exert its antitumor effect. This is supported by several findings that oxaliplatin-induced mechanisms of cytotoxicity, cellular responses, drug resistance and pharmacokinetics may be different from those induced by CDDP and carboplatin (39,58,59). Thus, these results further validate the notion that oxaliplatin could be an alternative treatment option for patients with OSCC with CDDP resistance, including those with p22phox overexpression. Furthermore, CDDP resistance in p22phox-ovexpressing OSCC tumors was demonstrated in a previous study by the authors, using the xenograft mouse model (19). Consistent with the *in vitro* results, while the antitumor efficacy of CDDP was significantly decreased in p22phox-overexpressing tumors, 5-FU could evidently inhibit tumor growth regardless of p22phox expression. Given that p22phox is commonly and abundantly expressed in OSCC cell lines and clinical tumors (18), the present results may explain why CDDP-based chemotherapy in combination with other non-platinum drugs such as 5-FU could give a better treatment outcome for oral cancer (15,35,60).

6. p22phox confers CDDP resistance by preventing CDDP access to the nucleus

To understand the molecular mechanisms by which p22phox conferred resistance to CDDP in OSCC, our group investigated how p22phox counteracted CDDP-induced apoptosis. It is well documented that the PI3K/Akt signaling pathway mediates suppression of apoptosis and promotes cell survival (61,62), urging us to test whether p22phox could enhance the activity of PI3K/Akt. While the p22phox-overexpressing OSCC cells exhibited increased PI3K/Akt activity and diminished CDDP-induced apoptosis, this drug-induced apoptotic signal was significantly restored when PI3K/Akt activity was inhibited by specific inhibitors or RNA interference (18). These results suggest that p22phox can counteract CDDP-induced apoptosis through the activation of the PI3K/Akt pathway, consistent with several previous reports that this signaling pathway is critical in promoting CDDP resistance in various

cancers (63-65). However, it remains to be elucidated how p22phox could regulate PI3K/Akt activity in OSCC cells. ER stress has been shown to markedly induce Akt activation, accounting for CDDP and doxorubicin resistance in liver and lung cancer cells, respectively (66,67). Since the ectopically expressed p22phox was co-localized with the ER and could induce ER stress in OSCC cells (unpublished data from our group), whether p22phox may activate the PI3K/Akt pathway via ER stress-dependent mechanism deserves further investigation.

Even a nearly complete suppression of PI3K/Akt activity by the specific inhibitors could not fully recover CDDP-induced apoptosis in p22phox-overexpressing OSCC cells, raising the possibility that additional mechanism(s) may contribute to this p22phox-dependent CDDP resistance. In a study by our group, it was observed that p22phox, when overexpressed, displayed a strong ring-like expression pattern at the nuclear periphery in both CDDP-resistant OSCC tumors and OSCC cell lines (18). It was hypothesized that p22phox-dependent CDDP resistance could involve this specific subcellular localization of p22phox. Fluorescence-labeled CDDP was used to monitor the distribution and abundance of CDDP in p22phox-overexpressing OSCC cells in an attempt to determine whether p22phox would impact CDDP uptake and trafficking in the cells. Whereas the fluorescence signal was uniformly distributed in the nucleus and cytoplasm of the control cells, the signal in the p22phox-overexpressing cells was mostly localized in the nucleus. Quantitative analysis revealed that the average cytoplasmic-to-nuclear ratio of the fluorescence intensity was overwhelmingly higher in p22phox-overexpressing cells compared with the control cells, suggesting blockade of CDDP nuclear entry following its normal uptake by the cells. More remarkably, it was found that the CDDP fluorescence signal was almost perfectly co-localized with the overexpressed p22phox at the perinuclear and other cytoplasmic regions. These results indicate that, after entering the cells, CDDP was sequestered in the cytoplasm by p22phox, thereby preventing its entry into the nucleus.

It is conceivable that blockade of CDDP nuclear entry leads to decreased DNA damage by the drug. Indeed, a significant reduction of CDDP-DNA adducts was observed in p22phox-overexpressing cells. In addition, activation of the checkpoint kinase 1-p53 signaling pathway, the DNA damage response during apoptosis elicited by CDDP-DNA adducts (68-70), was delayed and reduced throughout the treatment period of CDDP. Based on the above, a model of the mechanism of p22phox-dependent CDDP resistance in OSCC was proposed as follows. Despite normal uptake into the cell, CDDP access to the nucleus is markedly impaired due to sequestration by p22phox in the cytoplasm, thus resulting in decreased CDDP-DNA adduct formation and attenuated apoptosis. Subsequently, the diminished apoptotic signal was further inhibited presumably by the p22phox-activating PI3K/Akt pathway, ultimately leading to CDDP resistance in OSCC cells (Fig. 2). Overall, considering the mode and site of action, p22phox-dependent CDDP resistance appears to be a novel mechanism belonging to the category of pre-target resistance, the process occurring before binding of CDDP to its target, namely nuclear DNA (Fig. 1).

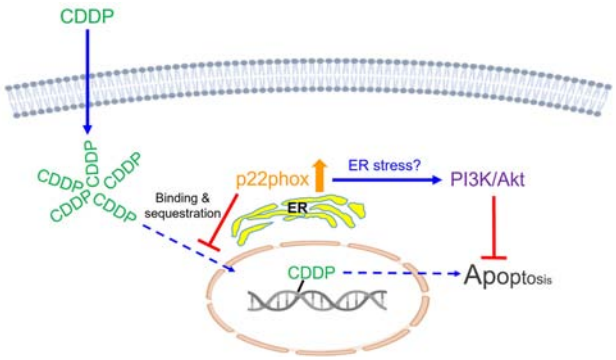

Figure 2. Mechanisms by which p22phox confers CDDP resistance in OSCC cells. Despite normal CDDP uptake into the cells, overexpression of p22phox in ER sequesters CDDP in the cytoplasm and blocks CDDP entry into the nucleus, thereby decreasing CDDP-DNA adduct formation and attenuating apoptosis. The diminished apoptotic signal is further inhibited presumably by p22phox- and ER stress-activating PI3K/Akt pathway, eventually leading to CDDP resistance in OSCC cells. Created with BioRender.com. CDDP, cisplatin; OSCC, oral squamous cell carcinoma; ER, endoplasmic reticulum.

7. Direct binding of p22phox to platinum drugs

Although studies by our group suggest that p22phox conferred resistance to CDDP by blocking CDDP nuclear entry in OSCC cells, the underlying molecular mechanism remained elusive. CDDP has been demonstrated to bind to numerous cellular proteins (71-73). Furthermore, it was observed that CDDP was almost perfectly co-localized with p22phox in the cytoplasm, motivating us to evaluate whether these two molecules could interact with each other. It was found that CDDP could bind to glutathione transferase (GST)-p22phox recombinant protein by GST pull-down assay, followed by co-immunoprecipitation, which further verified the CDDP-p22phox interaction in a cell model. In addition, using Tris-tricine SDS-PAGE and liquid chromatography-mass spectrometry, mapping of CDDP-binding sites in the p22phox protein revealed that CDDP could effectively bind to and even cross-link GA-30, a synthetic peptide fragment corresponding to a specific region in the cytosolic domain of p22phox protein. Furthermore, CDDP could interact with the GA-30 peptide fragment in a time- and dose-dependent manner, ensuring the binding affinity and specificity. Previous reports indicate three hot-spot amino acids, Cys, Met and His, with which CDDP could interact (74-76). It was then examined whether CDDP could bind to these amino acid residues in the GA-30 peptide. Amino acid substitutions at four hot-spot residues, Cys50, Met65, His72 or Met73, suggested that CDDP could potentially bind to all of the four residues in the peptide. However, there was differential binding selectivity and affinity of CDDP to the four amino acid residues, thereby contributing to different degrees to the CDDP-GA-30 interaction.

In addition, the impact of these potential CDDP-binding residues on p22phox-dependent CDDP resistance was demonstrated (17). Using site-directed mutagenesis, OSCC cell lines stably expressing the mutant versions of p22phox protein with point mutations at the respective four hot-spot residues were established. The results suggested that, compared to the wild-type version, p22phox protein with point mutations at Cys50, Met65 and Met73 markedly re-sensitized the

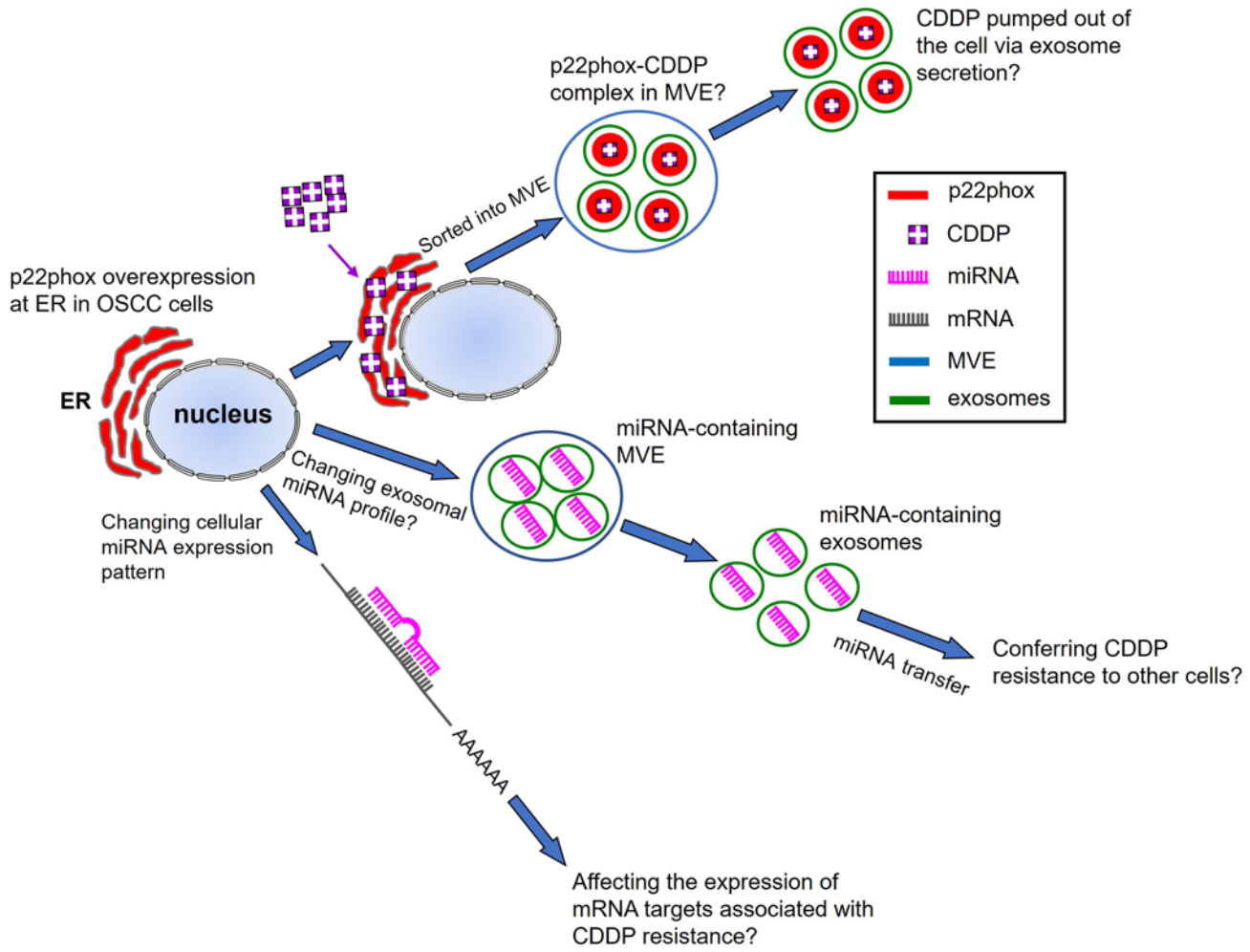

Figure 3. Future perspectives of p22phox-dependent CDDP resistance in oral cancer. The sequestered CDDP in the cytoplasm by p22phox binding could be shuffled into exosome biogenesis and trafficking pathway and eventually exported out of the cells via exosome secretion. In addition, p22phox overexpression could alter the expression profiles of cellular and exosomal miRNAs, which may confer CDDP resistance through exosome-mediated miRNA transfer and/or direct targeting of key mRNAs by the cellular miRNAs. ER, endoplasmic reticulum; MVE, multivesicular endosome; CDDP, cisplatin; miRNA, microRNA.

cells to CDDP-induced cytotoxicity and apoptosis, while the His72 mutation exhibited no such effect. However, it has yet to be determined whether simultaneous disruption of all the CDDP-binding sites in the p22phox protein can further sensitize the cells to CDDP treatment. Furthermore, the point mutation at His72 had little impact on CDDP-induced cytotoxicity and apoptosis, which is in agreement with the result that CDDP had the lowest binding affinity toward this amino acid residue in the GA-30 peptide. Whereas His72 has been a well-characterized polymorphic site (C242T) in p22phox correlated with the risk of coronary artery disease (76, 77), its role in chemoresistance is relatively obscure. Finally, to confirm the potential of p22phox to interact with other platinum and non-platinum drugs, a study by our group demonstrated that, in addition to CDDP, carboplatin and oxaliplatin could also bind to the GA-30 peptide. In sharp contrast, six non-platinum drugs, including 5-FU, docetaxel, etoposide, cytarabine, vincristine and daunorubicin, appeared to completely fail to interact with the peptide. These results indicate that p22phox may specifically interact with platinum but not non-platinum drugs. Of note, there was a sequence of increasing binding propensity to the GA-30 peptide: Oxaliplatin < carboplatin < CDDP, consistent with the aforementioned results that p22phox confers the same sequence of increasing resistance to platinum drugs.

Taken together, our group not only unprecedentedly reported the direct interaction between p22phox and small-molecule anticancer drugs, but identified yet another novel platinum drug-binding protein. More importantly, the findings by our group underscore the significance of this drug-protein interaction in drug resistance. Although several previous studies have demonstrated the ability of CDDP to interact with a plethora of cellular proteins (71-73), the role of such CDDP-binding proteins in drug resistance has remained largely elusive. A previous study revealed that silencing of a CDDP-binding protein, glutathione-S-transferase π, sensitized intrinsically resistant colon cancer cells to CDDP. Furthermore, inhibition of vimentin, another CDDP-binding protein, by a specific small-molecule inhibitor significantly enhanced the sensitivity to CDDP in CDDP-resistant ovarian cancer cells (79). However, how mechanistically these two proteins contribute to CDDP resistance has yet to be elucidated. As illustrated in Fig. 1, after entering the cell, CDDP may interact with cysteine-rich cytosolic proteins such as GSH and MTs, reducing CDDP activity and efficacy in patients with various cancers (43,80-82). However, both

GSH and MTs could act as a CDDP scavenger and sequester CDDP in the cytosol (42,83), typical examples of pre-target resistance (Fig. 1). It is not plausible that p22phox shares the same resistance mechanism with the two cysteine-rich proteins owing to differences in the amino acid composition and cellular function. Indeed, unlike GSH and MTs, p22phox is not a cysteine-rich protein, nor does it possess known detoxification enzyme activity. Thus, despite being categorized as the pre-target resistance, p22phox-dependent CDDP resistance still exemplifies a unique resistance mechanism against platinum drugs.

8. Conclusions and perspectives

In conclusion, based on the differential resistance to the platinum drugs and 5-FU, p22phox could not only be a prognostic biomarker that predicts chemotherapy outcomes, but also an indicator for alternative treatment strategies in oral cancer. Furthermore, findings by our group suggest a novel mechanism of platinum drug resistance in which p22phox binds to and sequesters the drugs in the cytoplasm, blocking the entry of the drugs into the nucleus and eventually leading to significantly reduced drug-induced cytotoxicity in OSCC. However, it remains to be proven whether this resistance mechanism is applicable to other types of cancer.

On the other hand, what could be the fate of the sequestered CDDP by p22phox? Preliminary studies by our group indicate that overexpression of p22phox may promote its own localization in and the release of exosomes from OSCC cells. Of note, previous reports suggest that, in CDDP-resistant OSCC and ovarian carcinoma cells, extracellular vesicles (EVs), such as exosomes, may carry and export CDDP out of the cells, at least in part contributing to the drug resistance (84,85). Therefore, it deserves investigation whether p22phox may carry the sequestered CDDP and then shuffle it into exosome biogenesis and the exosome secretory pathway, ultimately resulting in increased CDDP efflux and drug resistance. Since EVs play a crucial role in intercellular communication and there is mounting evidence that EV-based microRNA (miRNA) transfer confers resistance to CDDP in multiple cancer cells (86-89), it is speculated that p22phox-dependent CDDP resistance may also involve exosome-carried miRNAs. Indeed, the preliminary results suggest that p22phox overexpression drastically alters miRNA expression profiles in OSCC cells, including several differentially expressed miRNAs known to be associated with CDDP resistance. Thus, it remains to be elucidated whether such miRNAs have an impact on CDDP resistance through exosome-mediated intercellular communication and/or exosome-independent intracellular mechanisms, prior to regulating the expression of their target genes. Fig. 3 summarizes future research directions for p22phox-dependent CDDP resistance in OSCC.

Lastly, from the perspective of overcoming p22phox-dependent chemoresistance, in addition to adopting combinatorial therapeutic regimens as described above, p22phox appears to be a rational target for increasing the sensitivity of CDDP-resistant OSCC cells to CDDP. Thus, developing chemosensitizing agents that directly counteract the effects of p22phox is of fundamental importance. For instance, small-molecule inhibitors that specifically disrupt the p22phox-CDDP interaction may resensitize OSCC cells to CDDP treatment. Furthermore, since p22phox is abundantly expressed in CDDP-resistant OSCC tumors, nanoparticle-based delivery of miRNA or siRNA that specifically inhibit p22phox expression could potentially improve the efficacy of CDDP in future clinical validation.

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Availability of data and materials

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Authors' contributions

JYFC conceptualized the study. JCL, CYW and JYFC conducted the literature review and wrote the manuscript. THD, TJC,CCC and CHL provided critical opinions and revisions. CHL assisted with the language editing. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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