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# PERIOD1 (PER1) has anti-apoptotic effects, and PER3 has pro-apoptotic effects during cisplatin (CDDP) treatment in human gingival cancer CA9-22 cells

*Fuyuki Sato* [a,*], *Yunyan Wu* [a,b], *Ujjal Kumar Bhawal* [c], *Yang Liu* [a,b], *Tadaatsu Imaizumi* [d], *Satoko Morohashi* [a], *Yukio Kato* [e], *Hiroshi Kijima* [a]

[a] *Department of Pathology and Bioscience, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan*
[b] *Department of Pathology, College of Basic Medical Sciences, China Medical University, Shenyang 110001, China*
[c] *Research Institute of Occlusion Medicine and Open Research Center, Kanagawa Dental College, Yokosuka 238-8580, Japan*
[d] *Department of Vascular Biology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan*
[e] *Department of Dental and Medical Biochemistry, Hiroshima University Graduate School of Biomedical Science, Hiroshima 734-8553, Japan*

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ABSTRACT

PERIOD (PER) proteins are transcriptional regulators that are involved in circadian rhythms, sleep homeostasis, cell proliferation and tumour progression. We previously showed that the expression of PER1 was related to the regulation of apoptosis in human pancreatic cancer and hepatocellular carcinoma cells. However, the significance of PER in oral cancer has not been reported, and the detailed molecular mechanisms by which anti-tumour drug induces apoptosis in gingival cancer cells are not well understood. We examined whether PER1 and PER3 are involved in the regulation of apoptosis in human gingival cancer CA9-22 cells. The expression of PER1 and PER3 was upregulated and downregulated, respectively, by *cis*-diamminedichloroplatinum (II) (cisplatin: CDDP) treatment in CA9-22 cells, whereas CDDP treatment had little effects on the expression of PER1 and PER3 in human gingival fibroblasts (HGF-1). We found that short interference RNA (siRNA)-mediated knockdown of PER1 enhanced apoptosis of CA9-22 cells, and that PER1 regulated the amount of Bim, an apoptosis-related molecule. On the other hand, PER3 knockdown had an inhibitory effect on the apoptosis of CA9-22 cells induced by CDDP treatment. These results suggest that the alternation of expression of PER1 and PER3 was related to the apoptosis of CA9-22 cells. Furthermore, PER1 was intensely stained in the gingival cancer tissues, whereas PER3 was significantly stained in the non-tumour tissues by immunohistochemistry.

These findings suggest that PER1 and PER3 have anti-apoptotic and pro-apoptotic effects in human gingival cancer CA9-22 cells, respectively. The balance of PER1 and PER3 may modulate apoptotic reactions in gingival cancer cells.



## 1. Introduction

The frequency of oral cancer is about 3% of all cancers, and tongue and gingival carcinoma make up a high proportion of oral cancer cases. In addition, the incidence of oral cancer is increasing worldwide and so its diagnosis and treatment are critical issues.[1] Despite improvements in surgery, chemotherapy and radiation therapy, the overall 5-year survival rate for oral cancer remains at 50% and has not significantly improved in the past 30 years.[2,3]

* *Corresponding author:* Tel.: +81 172 39 5029; fax: +81 172 39 5030.
E-mail address: fsato@cc.hirosaki-u.ac.jp (F. Sato).
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*cis*-Diamminedichloroplatinum (II) (cisplatin: CDDP) is one of the most important anti-tumour drugs, and CDDP treatment induces DNA-damage and leads to apoptosis in various solid tumours.[4] CDDP treatment also affects transcription, translation, DNA repair and the cell cycle,[4–6] although some tumours are resistant to CDDP treatment. Several mechanisms are thought to be involved in the acquisition of resistance to CDDP. Tumours that are resistant to CDDP often show decreased drug accumulation and increased DNA-repair activity and transcription factor activity.[4,7] However, the details of the molecular mechanisms responsible for CDDP resistance are still unknown.

The induction of apoptosis is mainly regulated by the balance of pro- and anti-apoptotic factors. Bax, Bim, Bid and Bok are pro-apoptotic, while Bcl-2, Bcl-$X_L$ and Mcl-1, which belong to the Bcl-2 family, are anti-apoptotic proteins.[8] The overexpression of Bcl-2 and Bcl-$X_L$ is observed in the majority of oral cancers and correlates with chemotherapy resistance.[9,10] CDDP treatment has been shown to affect the expression of Bcl-2, Akt, X-linked inhibitor of apoptosis protein (XIAP), and Bim, and to activate the cleavage of caspases and poly (ADP-ribose) polymerase (PARP). CDDP treatment-induced apoptosis is also associated with p53 dependent and independent responses.[11] Bim is a Bcl-2 homology 3 (BH3)-only protein that has three isoforms (BimS, BimL and BimEL) and plays a critical role in initiating apoptosis in various cells.[12] The amount of Bim also correlates with apoptosis induced by various anti-tumour drugs, such as paclitaxel or gefitinib.[12,13]

PERIOD was first identified as a clock gene[14] and is known to have three isoforms (*PER1*, *PER2* and *PER3*). The three *PER* isoforms have similar structures[15,16] and work as transcriptional regulators of circadian rhythm; *PER1* and *PER2* are dominant negative regulators, while *PER3* is thought to be an output gene. Recently, it has been reported that PER1 was correlated with tumour progression.[17–21] However, a role of PER3 in tumours is poorly understood. PER1 has pro-apoptotic effects in prostate and colon cancers[22,23], whereas PER1 has anti-apoptotic effects in pancreatic cancer and hepatic carcinoma cells.[17] PER1 regulates apoptosis via p53-dependent or -independent responses. PER1 also regulates the expression of anti- and pro-apoptotic proteins in various tumours.[17,22] However, actions of PER in oral cancer cells have not been reported.

In the present study, we examined the effects of PER1 and PER3 on the apoptosis of human gingival cancer CA9-22 cells. PER1 knockdown enhanced the apoptosis induced by CDDP treatment and upregulated the expression of Bim. On the other hand, PER3 knockdown decreased the apoptosis induced by CDDP treatment. Our results suggest that PER1 and PER3 are anti-apoptotic and pro-apoptotic, respectively, in human gingival cancer cells.

## 2. Materials and methods

### 2.1. Cell culture and treatment

The human gingival cancer cell line CA9-22 was obtained from the Japanese Cancer Research Resources Bank. Normal human gingival fibroblasts (HGF-1) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These cells were cultured in Dulbecco's Modified Eagle's Medium-high glucose (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% foetal bovine serum at 37 °C in a humidified atmosphere of 95% air and 5% $CO_2$. In some experiments, the cells were incubated with CDDP (Sigma) at various concentrations for 24 h.

### 2.2. Human tissues

Well, moderately, or poorly differentiated human gingival cancer tissues ($n = 13$) were obtained at around 12:00 or 13:00 (Table 1). Histological specimens were retrieved from the archives of Hirosaki University Hospital under the guidelines produced by the Japanese Society of Pathology. We examined 11 surgical resection specimens and 2 biopsy specimens from 13 patients and analysed PER1 and PER3 protein expressions in the tumours and the adjacent non-tumour tissue.

**Table 1 – Immunohistochemical detection of PER1 and PER3 proteins in human gingival cancer tissues.**

| Cases | Age (year)/sex | Differentiation of squamous cell carcinoma | PERI protein expression | | PER3 protein expression | |
|---|---|---|---|---|---|---|
| | | | Tumour cells | Adjacent non-tumour cells | Tumour cells | Adjacent non-tumour cells |
| 1 | 66/M | Well | Strong | Weak | Weak | Strong |
| 2 | 80/F | Well | Strong | Weak | Strong | Strong |
| 3 | 80/F | Poorly | Strong | Weak | Strong | Strong |
| 4 | 75/F | Poorly | Strong | Weak | Weak | Strong |
| 5 | 85/F | Well | Strong | Weak | Weak | Strong |
| 6 | 85/F | Well | Strong | Weak | Weak | Strong |
| 7 | 53/M | Well | Strong | Weak | Weak | Strong |
| 8 | 59/F | Moderately | Strong | Weak | Weak | Strong |
| 9 | 76/F | Well | Strong | Weak | Weak | Strong |
| 10 | 62/M | Well | Strong | Weak | Weak | Strong |
| 11 | 52/M | Well | Strong | Weak | Weak | Strong |
| 12 | 70/M | Well | Strong | Weak | Strong | Strong |
| 13 | 68/M | Moderately | Strong | Weak | Weak | Strong |