Abstract. Background/Aim: The long noncoding RNA OIP5 antisense RNA 1 (OIP5-AS1) is overexpressed in various cancer types, such as lung cancer, hepatoblastoma and cervical cancer, and functions to accelerate cell proliferation, invasion and migration. Here, we investigated the role of OIP5-AS1 in cell-cycle progression of H1299 and A549 non-small cell lung cancer cells, and FaDu and CAL27 head and neck squamous cell carcinoma cells. Materials and Methods: The cells were transfected with small interfering RNA and subjected to cell-cycle analysis and reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Results: Silencing of OIP5-AS1 suppressed the proliferation of H1299, A549, FaDu and CAL27 cells. RT-qPCR and cell-cycle analysis revealed that silencing OIP5-AS1 increased the expression of CDK inhibitors, such as p15, p16, p18 and p19, resulting in G1-phase arrest. Conclusion: OIP5-AS1 regulates G1-phase progression by repressing CDK inhibitors and, thus, promotes the proliferation of H1299, A549, FaDu and CAL27 cells.

OPA-interacting protein 5 antisense transcript 1 (OIP5-AS1) is a long noncoding RNA (lncRNA) identified as the mammalian homolog of cyrano in zebrafish. In zebrafish, cyrano is essential for embryonic development (1). We previously showed that silencing human OIP5-AS1 inhibited the proliferation of HeLa cells by causing cell-cycle arrest at the G2/M phase, implying that human OIP5-AS1 functions to promote cell proliferation (2). Recent studies with clinical cancer specimens clarified that OIP5-AS1 is overexpressed in various cancer types such as of the breast, lung, cervix and bladder, as well as glioma, osteosarcoma and hepatoblastoma (3). OIP5-AS1 also participates in both positive and negative regulation of cell proliferation in cervical cancer (2, 4). Moreover, in many different human cancer cell types, including those of lung cancer (5), osteosarcoma (6), glioma (7) and hepatoblastoma (8), OIP5-AS1 functions to accelerate cell proliferation, invasion, migration and prevent apoptosis, so it is thought to function as an oncogene in these kinds of cancer. OIP5-AS1 acts as a sponge for many microRNAs (miRNAs) thereby inhibiting them, resulting in the regulation of WNT/β-catenin, PI3K/AKT serine/threonine kinase 1 and NOTCH signalling pathways (9).

Cell proliferation is strictly regulated by the cell cycle. The progression of the cell cycle is accelerated by a series of complexes consisting of cyclins and cyclin-dependent kinases (CDKs) and is repressed by CDK inhibitors (CKIs) (10). CKIs comprise two groups, the INK4 family including p15, p16, p18 and p19 and the Cip/Kip family including p21, p27 and p57 (11). Forced expression of these CKIs results in G1 phase arrest in the cell cycle. Regulation of the G1 phase by cyclin/CDK complexes and CKIs is thought to be important for the regulation of cell proliferation. Recently, we revealed that a lncRNA, antisense non-coding RNA in the INK4 locus (ANRIL), functions as a positive regulator of G1-phase progression in head and neck squamous cell carcinoma cells by repressing p15 and p16 expression (12). However, the involvement of OIP5-AS1 in cell-cycle regulation is largely unknown. In the present work, we studied the role of OIP5-AS1 in cell-cycle regulation in non-small-cell lung cancer (NSCLC) and head and neck squamous cell carcinoma cells.
Materials and Methods

Cell culture and RNA interference. H1299 and A549 human NSCLC cells, FaDu hypopharyngeal cancer cells, and CAL27 human oral cancer cells were acquired from the American Type Culture Collection (Frederick, MD, USA). These cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% foetal bovine serum (GIBCO, Grand Island, NY, USA). They were seeded at 5x10^5 cells in a 100 mm dish and cultured for 24 h, after which they were transfected with small interfering RNAs (siRNAs) using...
Lipofectamine RNAiMAX (Thermo Fisher Scientific, Waltham, MA, USA). The nucleotide sequence of siRNAs against OIP5-AS1 was 5’-GCAGCAUGCUGUGUGCAAA-3’ with 3’ dTdT overhangs.

The 3D culture of cells was performed as described previously (13). Briefly, 1,000 cells were seeded in a 96-well plate with an ultra low attachment surface and round bottom (Corning, NY, USA) and cultured for 72 h. Images of spheroids were then obtained under a CKX41 inversed microscope (Olympus, Tokyo, Japan). The spheroid areas were measured by Image J software.

Cell-cycle analysis. The cells transfected with siRNAs were collected at 72 h after siRNA transfection and fixed with 70% ethanol. The fixed cells were stained by a Muse® Cell Cycle Kit (Luminex, Toronto, Canada), in accordance with the manufacturer’s protocol and analysed using a Muse® Cell Analyzer (Merck Millipore, Darmstadt, Germany).

Reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The cells transfected with siRNAs were collected at 72 h after siRNA transfection and subjected to RT-qPCR as described previously (14). The nucleotide sequence of the primer set used for OIP5-AS1 was 5’-AAGCTGCCTTGAGAACTCAGAA-3’ and 5’-TGTCAGCAAATGCTGGACTCT-3’. The nucleotide sequences of the primer sets for p15, p16, p18, p19, p21, p27, p57 and glyceraldehyde 3-phosphate dehydrogenase have been described elsewhere (16).

Statistical analysis. The data in graphs are presented as the means and standard derivations of experiments were performed three times. The statistical analysis was carried out by two-tailed t-test. A
Figure 3. The effect of silencing of long noncoding RNA OIP5 antisense RNA 1 (OIP5-AS1) on expression of cyclin-dependent kinase inhibitors. H1299 (A), A549 (B), FaDu (C) and CAL27 (D) cells were transfected with small interfering RNA against OIP5-AS1 siRNA (siRNA) or control siRNA (Ctrl-i). At 72 h after siRNA transfection, the cells were collected. The expression levels of cyclin-dependent kinase inhibitors were measured by reverse-transcription quantitative polymerase chain reaction. The data are expressed relative to the corresponding value for the Ctrl-i cells. Significantly different at: *p<0.05, **p<0.01 and ***p<0.001; n.s.: not significant.
the proportion of cells in the G<sub>1</sub> phase and reduced that of H1299 and A549 cells in S and G<sub>2</sub>/M phases (Figure 2A-C). Silencing OIP5-AS1 also significantly increased the rate of FaDu and CAL27 cells in the G<sub>1</sub> phase (Figure 2D and E), similarly to the case in H1299 and A549 cells. These results suggested that OIP5-AS1 participates in the G<sub>1</sub>-phase progression in these cancer cells.

Given that silencing OIP5-AS1 caused G<sub>1</sub>-phase arrest, we next examined whether OIP5-AS1 participates in regulation of the gene expression of CKIs, which act as brakes on G<sub>1</sub>-phase progression. RT-qPCR data demonstrated that silencing OIP5-AS1 significantly increased the mRNA levels of p15 and p19 in H1299 cells (p16 was not detected) (Figure 3A) and that of p19 in A549 cells (p15 and p16 were not detected) (Figure 3B). Moreover, silencing OIP5-AS1 significantly increased the mRNA levels of p18 and p19 in FaDu cells (p16 was not detected) (Figure 3C) and those of p15, p16, p18 and p19 in CAL27 cells (Figure 3D). These results suggest that OIP5-AS1 participates in repressing the expression of CKIs such as p15, p16, p18 and p19.

Cancer cells have lost normal cell polarity and obtained the property of anchorage-independent growth through malignant transformation. Accordingly, 3D culture assays are suitable for studying the physiological proliferation of cancer cells. We finally examined whether OIP5-AS1 participates in the regulation of 3D proliferation. Silencing OIP5-AS1 significantly repressed the 3D proliferation of H1299 cells (Figure 4), suggesting that OIP5-AS1 promotes 3D proliferation.

Discussion

Some groups, including us, recently revealed that OIP5-AS1 participates in accelerating the proliferation of several types of cancer cell (2, 3). In the current study, we showed that silencing OIP5-AS1 caused cell-cycle arrest at the G<sub>1</sub> phase and suppressed the proliferation of H1299 and A549 human NSCLC cells, and FaDu and CAL27 head and neck squamous cell carcinoma cells. We also showed that silencing of OIP5-AS1 increased the expression of CKIs such as p15, p16, p18 and p19, which act as brakes on G<sub>1</sub>-phase progression in these cells. Taking these findings together, OIP5-AS1 appears to function to accelerate G<sub>1</sub>-phase progression by repressing p15, p16, p18 and p19, resulting in promotion of the proliferation of these cells. The mechanism of action by which OIP5-AS1 represses p15, p16, p18 and p19 is yet to be determined. However, it is known that OIP5-AS1 acts as a sponge for a few dozen miRNAs and competes against them (9). miRNAs participate in the post-transcriptional regulation of gene expression. Accordingly, OIP5-AS1 may repress the expression of p15, p16, p18 and p19 through its interaction with miRNAs. In future work, the involvement of miRNAs in the OIP5-AS1-mediated regulation of CKIs should be examined.
Recent study revealed that increased expression of OIP5-AS1 is observed in many types of human cancer, such as breast, lung, cervical and bladder cancer, glioma, osteosarcoma and hepatoblastoma (3), suggesting that OIP5-AS1 acts as an oncogene. However, the physiological relevance of OIP5-AS1 in head and neck squamous cell carcinoma is unclear and requires further study.

In conclusion, we propose that the increased expression of OIP5-AS1 leads to the promotion of G1-phase progression by repressing the expression of CKIs, resulting in tumour promotion.

Conflicts of Interest
The Authors declare no conflicts of interest directly related to the contents of this article.

Authors’ Contributions
Y.K., N.M., T.W. and R.O. conceptualized and designed the study, performed experiments and analysed data. Y.K. contributed to supervision of the study and wrote the article.

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