Polo-like Kinase Inhibitor Ro5203280 Has Potent Antitumor Activity in Nasopharyngeal Carcinoma

Arthur Kwok Leung Cheung¹, Joseph Chok Yan Ip¹, Hong Lok Lung¹,4, Jim Zhen Wu⁵, Sai Wah Tsao²,3,4, and Maria Li Lung¹,3,4

Abstract

Nasopharyngeal carcinoma is a cancer with its highest prevalence among the southern Chinese and is rare elsewhere in the world. The main treatment modalities include chemotherapy and radiotherapy. However, tumor chemoresistance often limits the efficacy of nasopharyngeal carcinoma treatment and reduces survival rates. Thus, identifying new selective chemotherapeutic drugs for nasopharyngeal carcinoma treatment is needed. In this current study, the antitumor efficacy of a polo-like kinase inhibitor, Ro5203280, was investigated. Ro5203280 induces tumor suppression both in vitro and in vivo. An inhibitory effect was observed with the highly proliferating cancer cell lines tested, but not with the nontumorigenic cell line. Real-time cell proliferation and fluorescence-activated cell sorting (FACS) analysis, together with immunohistochemical (IHC), immunofluorescence, and Annexin V staining assays, were used to evaluate the impact of drug treatment on cell cycle and apoptosis. Ro5203280 induces G2–M cell-cycle arrest and apoptosis. Western blotting shows it inhibits PLK1 phosphorylation and downregulates the downstream signaling molecule, Cdc25c, and upregulates two important mitosis regulators, Wee1 and Securin, as well as the DNA damage-related factor Chk2 in vitro and in vivo. In vivo tumorigenicity assays with Ro5203280 intravenous injection showed its potent ability to inhibit tumor growth in mice, with no observable signs of toxicity. These findings suggest the potential usefulness of Ro5203280 as a chemotherapeutic targeting drug for nasopharyngeal carcinoma treatment.

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Introduction

Nasopharyngeal carcinoma is a unique malignancy with an exceptionally high incidence in Southeast Asia, but it is rare elsewhere in the world. There are several cofactors that are believed to contribute to nasopharyngeal carcinoma development, including host genetics, Epstein–Barr virus (EBV) infection, and environmental factors. The loss of function of tumor suppressor genes (TSG) and loss of control of cell-cycle–regulating genes such as cyclin D1 (1) are important for nasopharyngeal carcinoma development. The nasopharynx is located deep inside the skull, making surgical excision a difficult first option line of therapy. Therefore, chemotherapy and radiotherapy are commonly used to treat patients with nasopharyngeal carcinoma. At present, the choices for chemotherapy in nasopharyngeal carcinoma are limited and chemoresistance often contributes to treatment failures. Therefore, there is a real need to identify alternative drugs for selectively targeting nasopharyngeal carcinoma.

In this current study, the drug Ro5203280 was tested and confirmed to inhibit nasopharyngeal carcinoma tumor cell growth. This drug mainly targets the polo-like kinase 1 (PLK1) protein. PLK1 is one of the well-studied members of the PLK family, which is an important regulator of different signaling pathways that are responsible for cell-cycle progression and regulation of mitosis (2).

PLK1 expression is commonly detected in the highly proliferating adult tissues, including the spleen and testis (3), and it serves as a marker of cellular proliferation (4). Overexpression of PLK1 was reported to associate with the high mitotic rate found in cancer (9).

The PLK1 protein plays various functional roles in G2–M cell-cycle control. It regulates the entry of cells into mitosis by phosphorylating the Weel protein and...
facilitating its degradation (10), and thereby activating the cyclin B/Cdk1 complex activity. Cdc25c regulates Cdk1 protein activation by removing the inhibitory phosphorylation of the Cdk1 protein (11). PLK1 also plays a role in the G2-M checkpoint. It is inhibited as a response to DNA damage-responsive ATM and ATR proteins (12). Moreover, PLK1 takes part in regulating the mitotic spindle formation by controlling the recruitment of γ-tubulin to centrosomes (13). PLK1 can also activate the anaphase-promoting complex (APC) to promote DNA segregation (14). This suggests that PLK1 plays an important role in cancer.

On the basis of the unique and important function of PLK1 in cancer, PLK1 has been molecularly targeted for cancer treatment. Interestingly, injection of a PLK1-specific antibody to transformed cells can induce mitotic arrest (13). Several PLK1 inhibitors, including B12536, B16727, Volasertib, GSK461364, and HMM-214, were used in clinical trials for colorectal cancer, NSCLC, and other type of solid tumors (15–17). Phase II clinical studies of B12536 have now been completed. In a recent study, an orally available PLK1-specific inhibitor was reported to induce cell-cycle arrest and apoptosis in cancer cell lines and xenograft tumor models (18). These earlier studies all suggest an important potential role of chemotherapeutic PLK1 inhibitors for targeted cancer treatment. Ro5203280 is a new PLK1 inhibitor that is now undergoing preclinical drug studies. Its structure is a close analog to BI2536, but has improved activity to PLK1. Therefore, more studies are warranted to investigate and confirm the functions of this new drug in inhibiting tumor growth.

In nasopharyngeal carcinoma, the function of PLK1 is still unclear and there are no reports to date on investigating the usefulness of PLK1 inhibitors in nasopharyngeal carcinoma chemotherapy. In this study, the function of the novel PLK1 inhibitor Ro5203280 was investigated. Results showed its efficacy in inhibiting nasopharyngeal carcinoma tumor cell growth in vitro and tumor formation in vivo by inducing cell-cycle arrest and apoptosis.

Materials and Methods

Cell lines

Three nasopharyngeal carcinoma cell lines (HONE1, HK1, and C666-1) and an immortalized nasopharyngeal epithelial cell line (NP460) were used in this current study. HONE1 and C666-1 were established from poorly differentiated nasopharyngeal carcinoma tumors, whereas HK1 was from a well-differentiated nasopharyngeal carcinoma. C666-1 is the only EBV-positive nasopharyngeal carcinoma cell line available for studies. NP460 is a normal nasopharyngeal epithelial cell line, which was immortalized by human telomerase. Culture conditions for these cell lines were as described previously (19–22). All cell lines used in the current study were tested and confirmed to be mycoplasma negative. They were obtained from the Hong Kong NPC AoE Cell Line Repository and have been authenticated using the AmpF/STR Identifier PCR Amplification kit (Life Technologies).

Drug preparation and Ambit selectivity screen

The Ro5203280 drug was provided by Roche Pharma Research and Early Development (23). The nocodazole was purchased from Sigma-Aldrich. For in vitro studies, Ro5203280 and nocodazole were dissolved in dimethyl sulfoxide (DMSO) solution. For the in vivo studies, the Ro5203280 was dissolved in a vehicle consisting of PEG400/acetate buffer (pH 5.0). The Ambit selectivity screen was conducted according to the manufacturer’s protocol (Ambit Biosciences).

Real-time cell proliferation assay

The real-time cell proliferation assay was conducted using the E-plate and the xCelligence System (Roche) according to the manufacturer’s instruction. In brief, a total of 1 × 10^5 HONE1, 1 × 10^5 HK1, 2 × 10^5 C666-1, and 2 × 10^5 NP460 cells were seeded onto an E-plate. Drug treatment was begun 24 hours after cell seeding. The cell proliferation index was automatically recorded by the xCelligence system computer program. All cell lines were treated with varying concentrations of Ro5203280 from 25, 50, 100, 200, 500, and 1,000 nmol/L or with DMSO, which is the in vitro vehicle control. The percentage of cell proliferation inhibition was calculated using the formula: % inhibition = 100 – (cell index of the treated well/cell index of untreated well) × 100% (24). All experiments were carried out in duplicate at least twice.

In vivo nude mouse tumorigenicity assay

The in vivo nude mouse tumorigenicity assay was conducted as described previously (25). All mice were kept in the Laboratory Animal Unit in the University of Hong Kong [Hong Kong (SAR), People’s Republic of China] according to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) international guidelines. In brief, a total of 1 × 10^7 cells of the tumorigenic nasopharyngeal carcinoma cell lines, HONE1 and C666-1, were injected subcutaneously into 2 groups of athymic BALB/c nude/Nu nude 6 to 8-week old nude mice. A total of 44 and 27 mice were used for the in vivo nude mouse tumorigenicity assay for HONE1 and C666-1 cell lines, respectively. Tumor sizes were measured weekly. After the tumors reached a size of 100 mm^3 or more, the drug was then injected into the mice intravenously. Drug concentrations of 15 mg/kg body weight (low dose) and 30 mg/kg body weight (high dose) were used for HONE1 treatment, whereas only the lower dose was used for C666-1. The drug was injected into the mice following a drug-dosing cycle regimen of 2 days on and 5 days off (2+/5−).

Cell-cycle and Annexin V apoptosis analysis

The cell-cycle distribution of the samples and Annexin V apoptosis assay were analyzed by FACScantoll (BD Bioscience) flow cytometry, as previously described (26). For the cell-cycle distribution of samples, in brief, a total of 1 × 10^6 cells was seeded on a T25 flask before treatment. The cell density of the untreated control reached more than 80% confluence before being subjected to the flow.
cytometric analysis. The cells were treated for 24 hours. A 200 nmol/L drug concentration was used for HONE1, HK1, and C666-1. A total of 10,000 stained cells were analyzed by fluorescence-activated cell sorting (FACS).

The Annexin V apoptosis assay was conducted by using the Annexin V, Alexa Fluor 647 Conjugate assay (Life Technologies) and nucleic acid staining dye, 7-aminoactinomycin D (BD Bioscience), according to the manufacturer’s instructions.

Immunofluorescence and immunohistochemical staining

The immunofluorescence and immunohistochemical (IHC) staining was conducted as previously described (27, 28). Antibodies specific for phosphorylated-Histone H3 and γ-tubulin were used for IHC and immunofluorescence staining, respectively (Table 1). The stained cells were observed under fluorescence microscopy (Nikon).

Western blot analysis

Western blot analyses of the PLK, phosphorylated PLK, and different signalling targets were conducted as previously described (26, 29). The protein cell lysates from the cell lines and from mouse tumors were extracted as previously described (24, 29). In brief, 1 × 10⁶ cells were seeded on a T25 flask before treatment. The cell density of the untreated control reached more than 80% confluence. The cells were treated for 24 hours at a 200 nmol/L drug concentration. The protein was then extracted by radioimmunoprecipitation assay solution supplemented with proteinase inhibitors. The antibodies and conditions used in this study are listed in Table 1. The α-tubulin was used as an internal loading control.

Statistical analysis

The real-time cell proliferation assay results represent the arithmetic mean ± range of experimental data. The SEM was used to calculate the SE of the in vivo assay. The Student t test was used to determine the confidence levels for group comparisons. A P < 0.05 was considered as statistically significant.

Table 1. Antibody information

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Results

Ro5203280 is a selective inhibitor for PLK1

Ro5203280 is a small-molecular PLK1 inhibitor that is structurally similar to BI2536 (30), but with an improved activity (Fig. 1A; ref. 23). In the Ambit protein kinase selectivity screen, a panel of 315 wild-type and mutant forms of kinases was investigated as described previously (31). Ro5203280 exhibited a high selectivity and showed a very low Kd (0.09 nmol/L) to PLK1 when compared with the 0.2 nmol/L Kd of BI2536. Although Ro5203280 does not bind to most of the protein kinases tested (Supplementary Table S1), for 8 protein kinases, it exhibits a Kd ranging from 70 to 2,000 nmol/L in the Ambit selectivity screen. The specificity of the PLK1 inhibitor Ro5203280 is summarized in Fig. 1B. Like BI2536, Ro5203280 also exhibits potent and broad activity in suppressing a large panel of 30 cell lines with IC₅₀ as low as 6 nmol/L (data not shown).

Ro5203280 inhibits in vitro cell and in vivo tumor growth

The efficacy of Ro5203280 in inhibiting in vitro cell growth was first investigated by the real-time cell proliferation assay. Comparisons of in vitro cell growth of nasopharyngeal carcinoma cell lines with the immortalized nontumorigenic nasopharyngeal epithelial cell line, NP460, showed EC₅₀ values for Ro5203280 of 78 nmol/L for HONE1, 193 nmol/L for HK1, and 358 nmol/L for C666-1, in contrast to 950 nmol/L for NP460 (Fig. 1C). The inhibitory effect decreases dramatically at drug concentrations less than 200 nmol/L. This shows that Ro5203280 can inhibit in vitro nasopharyngeal carcinoma cell growth.

The Ro5203280 is also able to inhibit in vivo tumor growth. In a previous Roche in-house study, different dosage levels and dosing regimes of Ro5203280 were tested to identify an effective in vivo dosage and schedule for Ro5203280 for different cancer cell lines, including the colorectal adenocarcinoma and small cell lung cancer (data not shown). Results suggested that the effective dosage is around 30 to 40 mg/kg with a 2+/5− dosing regimen by intravenous injection. Both
HONE1 and C666-1 cell lines are highly tumorigenic in nude mice (Fig. 2A and Table 2). For the HONE1 cell line, significant tumor growth inhibition can be observed with Ro5203280 treatment ($P = 0.002$) in both the low-dosage (15 mg/kg) and high-dosage (30 mg/kg) groups when compared with the vehicle control group, which was treated with PEG400/acetate buffer (pH 5.0). For C666-1, because a low dosage of Ro5203280 was found to induce significant tumor suppression in the in vivo tumorigenicity assay ($P < 0.0005$), the higher dose was not tested. The Ro5203280 does not show any acute toxicity in the mice, as assessed by mouse behavior and body conditions including hydration and changes of body weight (Fig. 2B) and gross necropsy. The effectiveness of the Ro5203280 in vivo levels was further confirmed by IHC staining with p-Histone H3 antibody. P-Histone H3 is a pharmacodynamic biomarker of PLK1 inhibition. Ro5203280-treated HONE1 and C666-1 tumors showed elevated p-Histone H3 levels (Supplementary Fig. S1). This finding further supports the potent role of Ro5203280 in nasopharyngeal carcinoma tumor inhibition.

**Ro5203280 induces cell-cycle arrest at G2–M phase by inhibiting mitotic spindle formation**

The tumor inhibitory mechanism for Ro5203280 was investigated. FACS analysis confirmed that 24 hours treatment of cells with 200 nmol/L Ro5203280 could induce cell-cycle G2–M arrest, as illustrated for HONE1 and HK1 (Fig. 3A). The percentage of cells in G2–M in HONE1, HK1, and C666-1 increased from 21.9% to 52.9%, 13.7% to 39.5%, and 15.4% to 19.6%, respectively, when compared with the vehicle control, whereas for NP460, no significant increase in the percentage of G2–M cells before and after treatment was observed. These results suggested that Ro5203280 has the ability to inhibit cell proliferation through G2–M arrest in HONE1 and HK1 cells. The Ro5203280-treated HONE1 and HK1 cells formed multinucleated cells (Fig. 3A and Supplementary Fig. S2). Ro5203280 induced cell-cycle arrest through inhibition of PLK1. The ability of treatment for 24 hours of 200 nmol/L Ro5203280 to induce mitotic spindle formation was investigated by 4', 6-diamidino-2-phenylindole (DAPI) and immunofluorescence staining. After DAPI staining, abnormal mitoses were observed in the Ro5203280-treated cells, providing evidence of nonuniform distributions of condensed chromatin (Supplementary Fig. S3A). All 3 nasopharyngeal carcinoma cell lines showed significantly increased populations of cells with abnormal mitoses (Supplementary Fig. S3B); this was not the case for NP460. This is consistent with the FACS analysis. Immunofluorescence staining results showed that the Ro5203280-treated HONE1 cells could selectively inhibit mitotic spindle formation when compared with the untreated and vehicle controls (Supplementary Fig. S2). A mitotic arrest inducer, nocodazole, was used as a mitotic arrest positive control. The Ro5203280-treated cells showed similar mitotic spindle inhibition effects as observed with nocodazole. These results indicate the role of Ro5203280 in inhibition of the mitotic spindle formation.

**Ro5203280 induces apoptosis in nasopharyngeal carcinoma cells**

The ability of Ro5203280 to induce apoptosis was quantified by Annexin V staining with FACS analysis. After

### Table 2: Summary of proteins

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<tr>
<th>Protein</th>
<th>Kd (nmol/L)</th>
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<tr>
<td>DAPK1</td>
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<tr>
<td>DAPK3</td>
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</table>

**Figure 1.** Inhibitory effect of Ro5203280 on NPC cell lines. A, chemical structure of Ro5203280. B, protein selectivity screen specificity of PLK1 inhibitor Ro5203280. C, in vitro growth inhibitory effect of Ro5203280 in 3 nasopharyngeal carcinoma cell lines (HONE1, HK1, and C666-1) and the immortalized nasopharyngeal cell line NP460. The relative inhibition rate was compared with their corresponding untreated control. The cells were treated with different concentrations of Ro5203280 (25, 50, 100, 200, 500, and 1,000 nmol/L) for 24 hours.
24 hours of 200 nmol/L Ro5203280 treatment, HONE1 cells showed a higher proportion of early apoptotic cells (20.8%), as compared with the untreated (3.77%) and vehicle controls (7.71%; Fig. 3B). In NP460, no significant increase in apoptotic cells could be observed in the Ro5203280-treated cells. This further supports the anti-apoptotic ability of Ro52035280 in cancer cells.

Ro5203280 inhibits PLK1 and its downstream target activities to inhibit tumor growth

Ro5203280 is a PLK1 inhibitor. The expression levels of PLK1 in the nasopharyngeal carcinoma cell lines were investigated. The PLK1 protein is upregulated in all 3 nasopharyngeal carcinoma cell lines when compared with NP460 (Fig. 4A). Phosphorylation levels of PLK1 were decreased in HONE1, HK1, and C666-1 cell lines treated with 200 nmol/L Ro5203280 (Fig. 4B). Postdrug treatment, levels of downstream proteins were determined. Wee1 and Securin, which are important molecules negatively regulating mitosis, were upregulated in the nasopharyngeal carcinoma cell lines treated with 200 nmol/L Ro5203280 (Fig. 4B). After treatment with 200 nmol/L Ro5203280, there was downregulation of the mitosis promoter, Cdc25c, in the nasopharyngeal carcinoma cell lines, HONE1 and HK1, but there was no significant change in the Cdc25c levels in C666-1 (Fig. 4B). The cyclin B protein level dropped after treatment with 200 nmol/L Ro5203280 in HONE1 and HK1 cell lines.

Table 2. Summary of Ro5203280 in vivo tumorigenicity assays

<table>
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<th>Cell lines/treatment description</th>
<th>Number of residual tumors after drug treatment/number of injection sites</th>
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<td>HONE1/Vehicle</td>
<td>16/16</td>
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<tr>
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<td>C666-1/Vehicle</td>
<td>10/10</td>
<td>–</td>
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<td>&lt;0.0005</td>
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*P value obtained by comparison with vehicle control.
lines, but this was not observed at the other Ro5203280 treatment concentrations. The protein levels of total Cdk1 did not show any significant differences after Ro5203280 treatment. Interestingly, one of the DNA damage checkpoint proteins, Chk2, seemed to have a higher phosphorylation level in nasopharyngeal carcinoma cells when treated with 200 nmol/L Ro5203280. This is suggestive that Ro5203280 may also be involved in regulating this signaling pathway.

After Ro5203280 treatment in vivo, tumor proteins were analyzed by Western blot analysis. Interestingly, phosphorylation of PLK1 was reduced in both HONE1 and C666-1 cell lines. Wee1, Securin, and phosphorylated Chk2 show upregulation in the Ro5203280-treated HONE1 and C666-1 tumors, whereas the Cdc25c and Cyclin B show downregulation (Supplementary Fig. S4). This further confirms the important role of Ro5203280 in nasopharyngeal carcinoma growth inhibition.

Discussion

Nasopharyngeal carcinoma is a deadly cancer that is usually diagnosed only at a late stage of cancer progression. Current treatment practice uses only limited standard anticancer drugs. With the development of drug resistance, curative treatment for patients with late-stage nasopharyngeal carcinoma remains a clinical challenge, and improved anticancer drugs are needed.

PLK1 overexpression is commonly observed in many different cancers (3, 5–7). PLK1 is the best characterized member in the PLK family, and increasing evidence suggests that PLK1 serves as a good candidate for cancer drug targeting in several malignancies; various PLK1 inhibitors are efficacious as anticancer drugs (18, 32, 33). A previous study showed that inhibition of PLK1 function suppresses cell growth and increases radiation sensitivity in medulloblastoma cell lines (34). In a diffuse large B-cell lymphoma study, a small-molecule PLK1 inhibitor, MLN0905, was confirmed to have anti-tumor effects (35). MLN0905 induces mitotic arrest and inhibits in vivo tumorigenicity (35). In recent clinical trial studies, a PLK1 inhibitor, BI2536, was used in a randomized phase II clinical trial of pancreatic exocrine adenocarcinoma (36). Furthermore, another PLK1 inhibitor, BI6727, was found to maintain broad antitumor activities in several solid tumors (37). However, BI2536 failed to show sufficient efficacy in phase II clinical trials; there was a low response rate and no significant difference in patient outcomes (36, 38). Ro5203280 is a close analog to BI2536, but with an improved activity against PLK1. These findings provide the rationale for evaluating the function of this novel PLK1 inhibitor in nasopharyngeal carcinoma. Ro5203280 holds promise as a novel molecularly targeted treatment drug for nasopharyngeal carcinoma.

In this current study, the effectiveness of Ro5203280 in selectively suppressing nasopharyngeal carcinoma growth, while sparing inhibition of the nontumorigenic NP460 cell line, shows its potential to serve as a chemotherapeutic agent. The 3 nasopharyngeal carcinoma cell lines displayed different EC50 values, reflective of the levels of expression of PLK1, the molecular target for Ro5203280. For NP460, which only expresses low levels of PLK1, Ro5203280 has little effect on its growth. On the other hand, PLK1 is expressed in the highly proliferative cancer cells and is regarded as a cell proliferation marker (3, 4).
levels were normalized to the internal control nasopharyngeal carcinoma cell lines. There is no significant change in the expression levels of Wee1, securin, and pChk2 is observed in the drug-treated nasopharyngeal carcinoma cells. The expression levels were normalized to the internal control α-tubulin. B, PLK, cell-cycle control, and DNA damage-related signaling pathways analysis of the Ro5203280-treated nasopharyngeal carcinoma cells. The expression levels were normalized to the internal control α-tubulin. The cells were untreated (U), vehicle (V), and drug treated (T), as indicated. The phosphorylation status of p-PLK1 in Ro5203280-treated cells with different concentrations of Ro5203280 is shown. Downregulation of cyclin B and Cdc25c and upregulation of Wee1, securin, and p-Chk2-T68 is observed in the drug-treated nasopharyngeal carcinoma cell lines. There is no significant change in the levels of Cdk1 protein in the Ro5203280-treated cells. The expression levels were normalized to the internal control α-tubulin.

These findings further support the potential efficacy of using this drug for treatment of nasopharyngeal carcinoma. The Ro5203280 compound does not induce any noticeable acute toxicity in the mice. However, due to inherent limitations in the use of mouse models, the primary hematologic toxicity seen in patients treated with other PLK1 inhibitors could not be studied but remains a potential side effect that warrants further future scrutiny.

PLK1 is involved in the cell-cycle G2–M transition control. The G2–M checkpoint is one of the important checkpoints for DNA damage repair and maintenance of genomic integrity (39). Normal cells suffering DNA damage do not undergo mitosis. If the damaged cells are unable to be repaired, they are destined to undergo apoptosis. On the other hand, inhibition of the mitosis can result in multinucleated cells. The multinucleated cells will finally undergo apoptosis. Therefore, the G2–M checkpoint is a good target for treating cancer (39). In cancer, the abnormal PLK1 activity can interfere with the normal G2–M checkpoint and thus, damaged cells will escape from the DNA repair mechanism and also cell-cycle control. Ro5203280 inhibits PLK1 kinase activity in cancer cells and induces G2–M arrest in the nasopharyngeal carcinoma cell lines. This finding helps to explain the low toxicity of Ro5203280 in the mice as the nontumor cells are spared.

PLK1 is a key regulator modulating different signaling pathways to control the G2–M transition. PLK1 induces Wee1 phosphorylation and thus, facilitates its degradation through proteasome-dependent degradation after ubiquitination by the E3 ubiquitin ligase (11). Wee1 is a crucial kinase in regulating the cell-cycle G2–M transition. It was confirmed to regulate DNA replication in human cells (40). It can phosphorylate Cdk1 and inhibit its function to drive cell-cycle progression (41). It can also induce apoptosis as observed during early xenopus embryonic development (42). Cdc25c removes the inhibitory phosphorylation of the Cdk1 protein and activates its functional role in mitosis (11). Early studies show that cyclin B was upregulated in the PLK1 knockdown nasopharyngeal carcinoma cell lines (8). However, in our current study, the cyclin B protein level decreased in the 200 nmol/L Ro5203280-treated cells; this may be related to cell apoptosis. At other Ro5203280 treatment concentrations, cyclin B protein was maintained at a high level.

In this current study, securin was found to be upregulated in Ro5203280-treated nasopharyngeal carcinoma cells. Securin is another key regulator for monitoring chromosome segregation, which is controlled by PLK1. PLK1 activates the APC function by degradation of APC inhibitors such as Eutil (43). The activated APC then degrades securin and releases the separase to allow chromosome segregation (44). The upregulation of securin by Ro5203280 also indicates its potential ability to cause G2–M arrest.

Furthermore, PLK1 plays a vital role for the G2–M recovery at the DNA damage checkpoint. Upregulation of phosphorylated Chk2 protein, which is crucial for the DNA damage response and induces cell-cycle G2–M arrest to allow DNA repair, was observed after Ro5203280 treatment. If the cell fails to repair the damage, it will undergo apoptosis (45). The DNA damage-induced cell-cycle arrest can inactivate PLK1 function. However, in cancer cells, PLK1 malfunction can override the G2–M arrest induced by DNA damage (46). PLK1 inactivates Chk2 kinase activity and thus, arrests the DNA damage checkpoint signaling (47). The inhibition of PLK1 function by Ro5203280 helps to maintain Chk2 function, allows the cell to undergo cell-cycle arrest, and prevents cell proliferation. Our results are further supported by a previous PLK1 study in nasopharyngeal carcinoma. Knockdown of PLK1 by siRNA in C666-1 and HK1 cell lines resulted in cell-cycle G2–M arrest, apoptosis, down-regulation of Cdc25c, and suppression of in vivo tumorigenesis (8). This further supports our findings of the potential usefulness of the PLK1 inhibitor, Ro5203280, to serve as a candidate chemotherapeutic drug for nasopharyngeal carcinoma.

Interestingly, our data show that C666-1 is more sensitive to Ro5203280 in vivo than in vitro. One possible explanation is that C666-1 grows relatively slowly in vitro.
but comparatively fast in the nude mouse. The changes in the molecular signaling pathways can help to explain differences observed in the efficacy of Ro5203280. In the Western blot analysis of C666-1 mouse tumors, down-regulation of the PLK1 downstream signaling target, CDC25C, is more obvious in Ro5203280-treated mouse tumors than in the in vitro study. Also the levels of upregulation of the Wee1 and Securin were much higher in the C666-1 mouse tumors than observed in vitro. This further supports our results that the Ro5203280 has a higher efficacy on C666-1 in vivo than in vitro.

In conclusion, the PLK1 inhibitor Ro5203280 shows a high efficacy for suppressing nasopharyngeal carcinoma growth with low toxicity in mouse studies. It suppresses nasopharyngeal carcinoma growth by inducing G2–M arrest and mitotic arrest and finally induces apoptosis through inhibiting PLK1 phosphorylation. It restricts the PLK1 function, then activates the signaling molecules that are negatively regulated by PLK1, and inactivates the molecules that are positively regulated by PLK1. Thus, this novel PLK1 inhibitor targeting multiple pathways is a promising anticancer drug for nasopharyngeal carcinoma treatment.

Disclosure of Potential Conflicts of Interest

S.W. Tsao has commercial research grant Roche Pharma Research and Early Development. M.L. Lung has commercial research grant from Roche Pharma Research and Early Development. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.K.L. Cheung, J.C.Y. Ip, M.L. Lung


Writing, review, and/or revision of the manuscript: A.K.L. Cheung, J.C.Y. Ip, H.L. Lung, J.Z. Wu, M.L. Lung

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.K.L. Cheung, J.C.Y. Ip, J.Z. Wu

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