·Basic Research·

Molecular mechanism of radiosensitizing effect of paclitaxel

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[**Abstract**] **Background & Objective**: Paclitaxel is a radiosensitizer which may stabilize microtubules, block the G_2/M phase of the cell cycle and thus modulate the radioresponsiveness of tumor cells. However, its potential molecular mechanisms of radiosensitization have not been well understood yet. This study was to investigate the radiosensitizing effect of paclitaxel on human oral epithelium carcinoma (KB) cell line and to explore the molecular mechanism of radiosensitization. **Methods**: The survival of KB cells following the treatment with paclitaxel and/or radiation was determined by colony-forming assay. The radiosensitizing effect was evaluated by calculating the sensitizing enhancement ratio (SER) with multi-target single hit model. The cell cycle distribution was analyzed by flow cytometry. Differentially expressed genes related to paclitaxel radiosensitization were screened using human Oligo microarray. Expressions of protein regulating cytokinesis 1 (PRC1) and cyclin B2 genes were confirmed by real-time quantitative PCR. **Results**: The proliferation of KB cells was significantly inhibited by paclitaxel combined with ionizing radiation. The $SERD_0$ and $SERD_q$ were (2.40 ± 1.87) and (12.23 ± 2.81) respectively, when the concentration of paclitaxel was 20 nmol/l. After the treatment with paclitaxel in combination with irradiation, the percentage of G_1 phase cells decreased from (48.32 ± 2.40)% to (15.73 ± 7.00)% ($P<0.01$), and the percentage of G_2/M phase cells increased from (13.66 ± 2.16)% to (52.51 ± 5.02)% ($P<0.01$). In total 176 differentially expressed genes were identified to be related to paclitaxel radiosensitization. Ten genes were found to regulate cell division, two of which were up-regulated and eight were down-regulated after the treatment. Moreover, the expression of PRC1 and cyclin B2 was decreased. **Conclusion**: The radiosensitizing effect of paclitaxel on KB cells may be due to the down-regulated expression of PRC1 and cyclin B2, resulting in inhibition of mitotic spindle formation and cell necrosis.

Key words: radiosensitization, paclitaxel, KB cells, cell cycle, microarry, fuorescent quantitation PCR, protein regulating cytokinesis 1 (PRC1), cyclin B2

Paclitaxel is a radiosensitizer which is generally used in radiotherapy of clinical oncology. Paclitaxel can enhance the radiosensitivity of tumor cells.[1,2] The mechanism is considered that palitaxel binds directly to both microneme and microtubule, inhibits depolymerization at kinetochore microtubule, prevents chromosomal migration and depolarization, which would result in cell arrest in radiosensible G_2/M phases.[3] However, it was reported

that both paclitaxel and ionizing radiation could induce tumor cells to produce multinucleated giant cells throught acting on the mitotic spindle checkpoint, so that normal mitosis progression could not be sustained, which is not unrelated to microtubule inhibition.[4] It is even considered that tumor cells escape from apoptosis through the formation of multinucleated giant cells and become resistant, direct affecting the effectiveness of radiation therapy and chemotherapy.[5] In this study, we performed genetic screening using GeneChip at the molecular biology level, to identify genes related to radiosensitization and try to clarify the mechanism of the radiosensitizing effect of paclitaxel, thus provide some theoretical basis for oncotherapy and drug research.

Materials and Methods

Reagents and equipments. The following reagents and equipment were used : paclitaxel (Sichuan Chaohui biotechnology company, China); RPMI-1640 culture medium and newborn calf serum (Gibco, USA); six-well adherent cell culture plates (Corning); Genechip Human-6_V2 (Illumina); Tiizol (Invitrogen); Primescipt RT reagent kit (TakaRa, China); real-time quantitative PCR kit, 2× SYBR Green PCR Master Mix (ABI); HERA Carbon dioxide incubator (Kedro, USA); inverted microscope CK40-F200 (OLYMPUS); linear accelerator KD-2 (Siemens); Flow cytometer FC-500 (Beckman); ScanArray4000 (Gsi Lumonics, USA); real-time quantitative PCR detector PRISM7000 (ABI).

Cell culture and morphology observation. Human oral epidermoid carcinoma KB cell line was purchased from Shanghai cell bank, Chinese academy of sciences, and was cultured in RPMI-1640 medium containing 15% newborn calf serum, supplemented with 15 mmol/ L HEPES, 2.2 g/ L sodium bicarbonate, 2 mmol/ L glutaminate, at 37℃, 5% CO2. Cells were passaged every three days. Cells were taken photographs before and after the treatment within 120 h under the inverted microscope.

Clone formation assay. KB Cells during logarithmic phase of growth were digested with trypsin to prepare a single cell suspension, and seeded into six-well plate at different densities for different treatments. In preliminary experiments, the cell adherent rate was about 30% in the six-well plate, and the cell number was hard to count at a density of 1000 per well in the blank group. No occurrence of extreme status in the highest dose group of 8Gy was observed, at a density of 50 clones per well or above, indicating a certain radio-resistant capacity of KB cells. Therefore, 800 cells per well were seeded in the X-ray alone group. Clone formation was decreased evidently after the treatment with paclitaxel, therefore cells were added at the density of 1.0×10^4, 2.5×10^4 and 5.0×10^4 per well with concentrations of paclitaxel of 5, 10, 20 nmol/L.

After adherence, cells were cultured continuously for 20 h and changed to fresh medium containing different concentrations of paclitaxel, and then irradiated with the linear accelerator at a dose rate of 200cGy/ min, a source-skin distance of 100 cm, and irradiation doses of 0, 2, 4, 6, 8 Gy. Drugs were immediately removed after irradiation. Cells were washed twice with PBS, and fresh medium without drugs was added for continuous culture for nine days. Medium was changed every three days. After nine days of culture, cells were fixed with 95% alcohol, stained with Giemsa. The number of clones consisting of more than or equal to 50 cells was counted. The cell survival fraction (SF) was recorded as follows:

Utilizing Sigma Plot 2001 Demo software, the cell survival curve was plotted based on the multi-target single hit model , $Dq = Do \cdot 1nN$, and parameters of radiosensitivity for each group were determined. D_0 represented the mean lethal dose, Dq represented the quasi-threshold dose, SERDo or SERDq represented the ratio of Do or Dq of the control group to Do or Dq of the experimental group.

Cell cycle distribution measured by flow cytometry. Cells were divided into four groups: control group, paclitaxel group, X-ray group and paclitaxel plus X-ray group. Prepared cell suspensions were seeded into culture flasks, with 1×10^5 cells/ml. After growth of adherent cells,

the paclitaxel group and the paclitaxel plus X-ray group were treated with 40nmol/L paclitaxel, 20 h later, the paclitaxel plus X-ray and the X-ray groups were treated with 6 Gy irradiation, and cells were collected after 10 h. Cell suspensions were collected into a 5 mL test tube, centrifuged for 10min at a speed of 1000× g, washed twice with PBS, added with 10ml of staining solution PI (20μ g/mL) and 500μ L of Binding buffer to react for 30 min in dark, and then quantitatively detected by flow cytometry.

Genechip assay. Cells were divided into groups as mentioned above, and total RNA was extracted using Trizol reagent. The absorbance value was detected at 260nm and 280nm wave length using a 752 UV spectrophotometer. The purity and concentration of RNA were assayed. Pure cRNA was obtained using the IlluminaTotalprep Kit, stained with Streptavidin-Cy3 and then mixed with the hybridization mixture, sampled to the chip, hybridized at 55℃ for 16 h, scanned with the chip scanner after washing the films. Images were saved as the grayscale tiff file. Using ScanAlyze software, lattice of the grayscale tiff file was transformed into digital data, and the initial data were calculated by removing background and compared. Data in the chip was normalized using the Cubic Spline method, and differentially expressed genes were screened related to radio-sensitization.

Real-time quantitative PCR. PRC1 and Cyclin B2 genes highly related to cell division were selected from the differential genes related to radiosensitization, detected by real-time quantitative PCR to conform the results of genechip, and GAPDH was used as control.

RT-PCR. Total RNA was extracted using Trizol, and the quality and concentration of RNA were detected using the same method used in Genechip. cDNA synthesis by RT-PCR was performed with primescirpt RT Enzyme mix I (TakaRa), 1ug of total RNA was used for each group, Oligo dT as primers.

Primer design and synthesis. According to the gene sequence of PRC1, Cyclin B2 and GAPDH in NCBI database, real-time PCR primers were designed with Oligo 6.0 and performed by TaKaRa company. The primers were as follows: PRC1 (upstream) 5-CTCCAGCGCAACTTCAGCATTA-3, (downstream) 5-GCCCAACAGTGGAACTGTCAGA-3, with the product size of 91bp; CyclinB2 (upstream): 5-TGCAGCACATGGCCAAGAA-3, (downstream): 5-CTTCAGGAGTTTGCTGCTTGCATA-3, with the product size of 95bp; GAPDH (upstream) 5-GCACCGTCAAGGCTGAGAAC-3, (downstream) 5-TGGTGAAGACGCCAGTGGA-3, with the product size of 138bp.

Real-time quantitative PCR. The PCR reaction system (25μ L) was performed (2× SYBR Green PCR Master Mix 12.5μ L, 50μ mol/L upstream and downstream primers, for each 0.5μ L, cDNA 1μ L). The PCR procedure was as follows: 50 ℃ 2 min, 95 ℃ 10s; then 95 ℃ 15 s, 60 ℃ 1min, for 40 cycles. Melting curve was begun to be analyzed from 60 ℃. GAPDH and target genes were placed in the same plate under the same condition, and a blank control was set for each reaction. The results were assayed with the method of Threshold cycle (CT), which compared the fluorescence signals reflecting the cycle number of the exponential growth point to that of the background during PCR amplification. House-keeping gene GAPDH was used as control to quantify the relatively level of the target gene. The calculation formulas were as follows:

Volume of target gene expression of experimental sample $=2^{-\triangle\triangle CT}$

$\triangle\triangle C_T = \triangle C_T$ of experimental sample $- \triangle C_T$ of control sample

$\triangle C_T = C_T$ value of target gene $- C_T$ value of control gene

Statistical analysis. Values are expressed as± s. One-factor analysis of variance was used to compare Do, Dq values of each group cells and expression of PRC1 and CyclinB2 was performed. χ^2 test was adopted to calculate the distribution ratio of cells at each phase. $p<0.05$ was considered statistically significant.

Results

Cell morphologic change. A great amount of polykaryocytes were found after the treatment with paclitaxel and paclitaxel plus X-ray, and were increased as time prolonged within a certain period of time. The process is shown in Fig.1: cells were changed from a single nucleus to a great quantity of polykaryocytes after the treatment with paclitaxel and paclitaxel plus irradiation, and to cell disruption and cell death in the end.

Radio-sensitization effects. Taken Do, Dq value as the standard, the radio-sensitization ratio was calculated and the results are shown in Table 1. The Do value was statistically significant only when the concentration of paclitaxel was at 20nmol/L ($p<0.05$), and the Do value showed difference when the concentration of paclitaxel was at 5nmol/L above ($p<0.05$). The radio-sensitization effect became obvious when the concentration of paclitaxel was at 20nmol/L. The cell survival curve with the treatment of different concentrations of paclitaxel is shown in Fig.2.

Cell cycle distribution. Changes in cell cycle distribution and the apoptotic rate are shown in Table 2. Cell phases in the X-ray were rarely different compared with those of the control group; in the paclitaxel and paclitaxel plus X-ray groups, both cell proportions at the G1 phase were decreased, while the proportion at the G^2/M phase were both increased, but the differences were no statistically significant between these two groups; the cell ratio of S phase was decreased in the paclitaxel plus X-ray group, but was still higher than that in the paclitaxel group, and the difference was significant between these two groups ($p<0.05$); apoptotic rates were increased in both the paclitaxel and paclitaxel plus X-ray groups, however, the ratio was lower in the paclitaxel and X-ray group compared with that in the paclitaxel group. Significant changes were

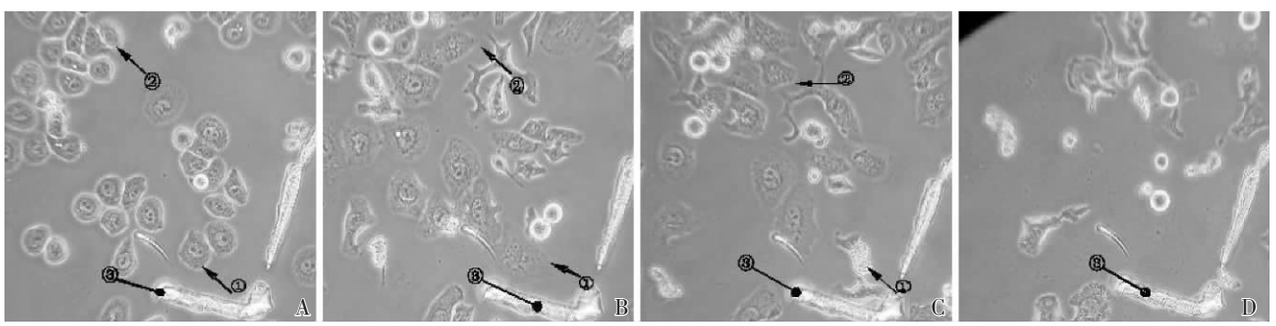

Figure 1 Process of multinuclear giant cell formation and death induced by paclitaxel and radiation. (×200)
A–D: Treatment with paclitaxel and radiation for 20, 40, 50 and 70 h, respectively. Arrow ① shows the process of a cell from one nucleus (in A) to a multinuclear giant cell (in B) and finally being disintegrated (in C). Arrow ② shows the same process of another cell. Arrow ③ is the scratch reference.

Table 1 Relative biological parameters and sensitive enhancement ratio of different paclitaxel groups

Drug concentration (nmol/L)	D_o	D_q	$SERD_o$	$SERD_q$
0	2.32±0.44	4.57±0.07	–	–
5	2.85±0.35	3.62±0.56[a]	0.82±0.11	1.28±0.19
10	2.10±0.51	2.72±0.84[a]	1.12±0.09	1.79±0.57
20	1.49±0.71[a]	0.44±0.08[b]	2.40±1.87	12.23±2.81

[a]$P<0.05$, vs. 0 nmol/L group; [b]$P<0.01$, vs. 0 nmol/L group. All results are presented as mean±SD of three independent experiments.

Table 2 Cell cycle distribution and apoptotic rate of different groups

Group	G_1(%)	G_2/M(%)	S (%)	Apoptosis(%)
Control	48.32±2.40	13.66±2.16	38.02±0.94	1.35±0.61
Paclitaxel only	28.35±5.30[a]	52.17±2.67[a]	15.45±3.07[a]	29.91±5.60[a]
Ratiation only	50.09±0.93	17.51±6.19	32.40±5.26	2.09±1.54
Paclitaxel and radiation	15.73±7.00[a]	52.51±5.02[a]	30.26±0.15[ab]	11.78±0.49[ab]

[a]$P<0.01$, vs. control group; [b]$P<0.05$, vs. paclitaxel only group. All results are presented as mean±SD of three independent experiments.

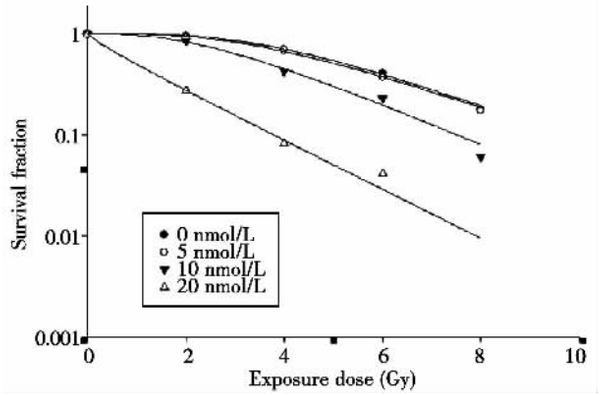

Figure 2 Survival curves of KB cells after the treatment with different concentrations of paclitaxel in combination with radiation

Table 3 Categorization for gene functions related with radiosensitization

Function	Number	Down-regulated	Up-regulated
Cell division	10	8	2
Apoptosis	4	1	3
DNA synthesis, repair protein	4	3	1
DNA binding, transcription factor	15	7	8
Cell receptor	5	2	3
Immunity	3	2	1
Cell signal and transmission protein	30	21	9
Metabolism	20	16	4
Development	4	4	0
Other	82	57	25

observed in the paclitaxel and paclitaxel plus X-ray groups compared with the control group; changes in the paclitaxel group were mainly an decrease in cells in the S phase and an increase of cells in the G2/S phase, while those in the combination group was a decrease of cells in the G1 phase.

Gene expression variance. The screening standard of different genes was that with the effect genes either in the experimental or control group, the diffscore of the experimental group was less than -20 or more than 20, using Oligo chip containing 48000 human genes. Compared with the control group, there were in total 750, 1030 and 851 differentially expressed genes in the X-ray group, paclitaxel group and paclitaxel plus X-ray group. Using the intersect method, the commonly differentially expressed genes in the X-ray group and the paclitaxel group were removed from 851 genes in the paclitaxel plus X-ray group, , and finally 176 specific expression genes related to radio-sensitization were obtained. There were 63 down-regulated genes and 31 up-regulated genes (Table 3). Ten groups were divided based on the gene function, and there were 10 genes related to cell division, of which two genes were up-regulated and eight genes were down-regulated (Table 4).

Specifically expressed genes. After screening of differentially expressed genes related to radio-sensitization, 10 genes related to cell division were analyzed. Cyclin B2 and PRC1 which are related to mitosis anaphase were selected for quantitative analysis. As shown in

Table 4 Differentially expressed 10 genes related with mitosis

ACCESSION	Gene name	Difference score		
		1vs4 [a]	1vs2	1vs3
NM_004701	NM_004701 CYCLIN B2	−31.0	−7.6	−17.9
NM_003981	PROTEIN REGULATOR OF CYTOKINESIS 1 (PRC1)	−22.4	−4.9	−13.9
NM_001012271	NM_001012271 BACULOVIRAL IAP REPEAT-CONTAINING 5 (SURVIVIN)	−20.1	8.5	−16.2
NM_006035	NM_006035 CDC42 BINDING PROTEIN KINASE BETA (DMPK-LIKE)	−27.4	−7.2	−18.8
NM_002824	NM_002824 PARATHYMOSIN	−24.9	−17.1	−1.6
NM_015089	NM_015089 P53-ASSOCIATED PARKIN-LIKE CYTOPLASMIC PROTEIN	−31.0	−7.6	−19.9
NM_033215	PROTEIN PHOSPHATASE 1, REGULATORY SUBUNIT 3F	−24.1	−13.5	4.2
NM_031299	CELL DIVISION CYCLE ASSOCIATED 3 (CDCA3)	−37.7	−3.5	−6.7
NM_002829	PROTEIN TYROSINE PHOSPHATASE, NON-RECEPTOR TYPE 3	20.6	8.6	3.4
NM_024063	SPERMATOGENESIS ASSOCIATED 5-LIKE 1	21.7	18.0	6.8

[a]Difference score between different groups: 1. control; 2. paclitaxel only; 3. X-ray only; 4. paclitaxel and X-ray.

Fig.3, the quantitative PCR melting curve indicated that PCR amplification was specific and the results were credible for the unimodal of GAPDH, PRC1 and Cyclin B2. Gene expressions of Cyclin B2 and PRC1 were both decreased in the paclitaxel group, X-ray group and paclitaxel plus X-ray group, with were most obvious in the combination group. PRC1 and Cyclin B2 genes were down-regulated to (0.35± 0.06) and (0.52± 0.06) of that of the control group, and the differences were statistically significant ($p<0.05$), which were coincident with the genechip results. Gene expressions of PRC1 and Cyclin B2 were not statistically significant among the paclitaxel group, the X-ray group and the control group (Table.5).

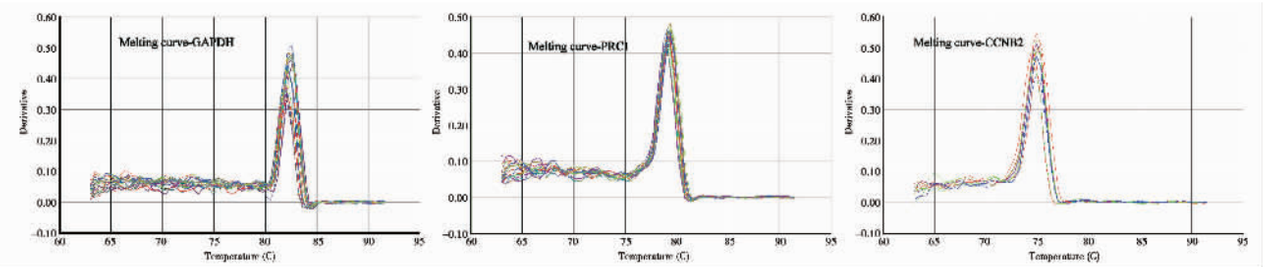

Figure 3 Melting curves of GAPDH, PRC1 and CCNB2

Table 5 The mRNA expression level of PRC1 and CYCLIN B2

Group	PRC1			Cyclin B2		
	ΔC_T	$\Delta\Delta C_T$	fold	ΔC_T	$\Delta\Delta C_T$	Fold
Control	5.35±0.28	–	1.00	3.11±0.31	–	1.00
Paclitaxel only	6.00±0.80	0.89±0.33	0.55±0.14	3.18±0.30	0.07±0.11	0.96±0.07
Radiation only	6.37±0.86	1.25±0.41	0.43±0.13	3.59±0.13	0.48±0.18	0.72±0.09
Paclitaxel and radiation	6.53±0.74[a]	1.41±0.34	0.38±0.09[a]	4.07±0.24[a]	0.96±0.18	0.52±0.06[a]

[a]$P<0.05$, vs. control group. All results are presented as mean±SD of three independent experiments.

Discussion

Radiotherapy is an important means for oncotherapy in clinical treatments. Approximately 70% -80% of the cancer patients are in need of radiotherapy. To achieve better therapeutic effects, chemotherapeutics are often used in clinic to increase the sensitivity of radiotherapy. Paclitaxel is generally used in oncotherapy. Previous study showed that paclitaxel binds to microneme and microtubule to promote microtubule polymerization and inhibit kinetochore microtubule depolymerization, which prevents chromosome migration and depolarization to cause arrest of cells in the G_2/M. 6 Cells with chromatid in the G_2/M phase are most sensitive to radiation damage, therefore, it is considered that the radiosensitizing effect of paclitaxel is caused by the arrest of G_2/M phase cells that are radiosensitive.

This study confirmed that paclitaxel indeed increased radiosensitivity of tumor cells. After the treatment with paclitaxel and irradiation, the proportion of cells in the G_2/M phase was increased obviously, with a great quantity of disaggregated polykaryocytes which were dead in the end. Although paclitaxel could lead to an increase in cells at G_2/M phase, its overall regulation on the cell cycle distribution is different from that of paclitaxel plus X-ray. Cells mainly trended from S phase to G_2/M phase treated with paclitaxel only, while from G_1 phase to G_2/M phase with paclitaxel and X-ray. Radiobiology research indicated that tumor cells in the G_1/S phase radiated by X-ray entered and remained in the G_2/M phase, which is an important way to increase radiosensitivity and

improve the therapeutic effect.[7] From the cell cycle distribution results, the apoptotic ratio of paclitaxel combined with irradiation was lower than that of paclitaxel, and it was supposed that the mechanism of the radiosensitizing effect of paclitaxel was related to the direct cell death pathway resulted from paclitaxel combined with irradiation rather than the apoptotic pathway.

To further study the molecular mechanism of radiosensitizing effect of paclitaxel on cell cycle, 10 differentially expressed genes were screened using Genechip. Cyclin B2, PRC1 and Survivin genes related to cell division were detected decreased obviously, while the function of two up-regulated genes, PTPN3 and SPATA5L1, were undefined. Cyclin B2 and PRC1 genes related to mitosis anaphase were selected for quantitative analysis, and the results showed that the expressions of Cyclin B2 and PRC1 were deceased obviously compared with the control. Inhibition of these three genes affected cell division.

To successfully pass through all phases of the cell cycle, multi-factors are needed to co-regulate, in which Cyclin plays a key role. CyclinB1 and CyclinB2 are the main two types of CyclinB in mammals. CyclinB is synthesized firstly in the S phase, and its concentration is increased while cells enter the G_2 phase.[8] Cyclin B2 is necessary in the course of bipolar spindle formation.[9] In this study, Cyclin B2 was significantly decreased after the treatment with paclitaxel, and cells were unable to form the bipolar spindle, and remained in G_2/M phase, resulting in abnormal cell division. These indicated that paclitaxel could increase radio-sensitization of tumor cells by inhibiting the expression of Cyclin B2.

Protein regulator of cytokinesis (PRC) 1 is a 71 KD CDK substrate protein. It can bind to microtubule and form fasciculation. Phosphorylation of PCR1 by CDK1/Cyclin B1 is the first step in the formation of spindle fasciculation.[10] Previous siRNA experiments confirmed that the loss of PRC1 resulted in abnormality of chromosome arrangement and separation, and an increase in dikaryon cells.[11,12] Down-regulation of PRC1 gene expression by paclitaxel and irradiation resulted in dikaryon and even polykaryocytes, and cell death in the end.

Survivin is the most strong inhibitor of apoptosis at present, with its direct effect on the end phase of the process that cytochrome C is released from mitochondria to enter into the cytoplasm, thus to inhibit Caspase3/7 activity.[13] Additionally, Survivin has influence on the stability of kinetochore microtubule as well as its annex and signaling transduction of the spindle checkpoint, to participate in normal cell division. ¼ Cells were arrested in the G_2/M phase with RNA interference target Survivin.[15] Down-regulation of survivin resulted in G_2/M arrest after the treatment with paclitaxel combined with irradiation. Relationship among those three genes and cell division is summarized in Fig. 4.

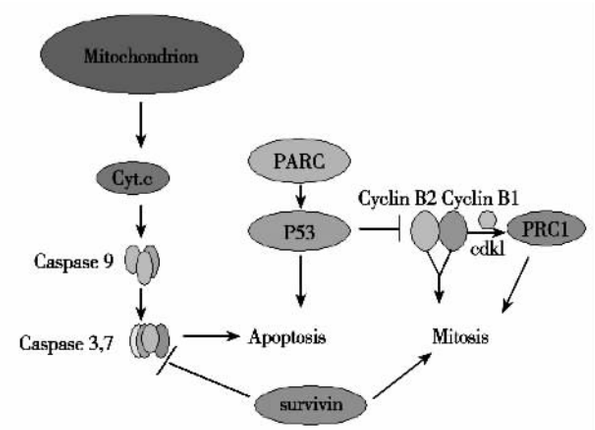

Figure 4 Possible relationship among PRC1, Cyclin B2 and survivin in cell mitosis

In summary, the radiosensitizing effect of paclitaxel at least includes two aspects: the first is cell transition from the G1 phase to the G_2/M phase by altering cell cycle distribution, which results in formation of polykarycytes and cell death rather than apoptosis; the second is inhibition on spindle formation caused by the down-regulation of PRC1 and Cyclin B2, which results in abnormal cell division, formation of polykarycytes, cell disaggregation, finally leading to cell death.

References

[1] Sarhan S, Kayisogullari U, Ercan I, et al. Randomized phase 2 study of radiotherapy alone versus radiotherapy with paclitaxel in non-small cell lung cancer [J]. J Int Med Res, 2004,32(4):375-383.

[2] Xie GQ, Liao GQ, Li LL, et al. Clinical study of paclitaxel in the radiotherapy of nasopharyngeal carcinoma[J]. Yao Pin Ping Jia, 2007,4(2):117-118. [in Chinese]

[3] Elomaa L, Joensuu H, Kulmala J, et al. Squamous cell carcinoma is highly sensitive to taxd, a possible new radiation sensitizer [J]. Acta Otolaryngol, 1995,115(2):340-344.

[4] Long BH, Fairchild CR. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase [J]. Cancer Res, 1994,54(16):4355-4361.

[5] Kim SM, Kim R, Ryu JH, et al. Multinuclear giant cell formation is enhanced by down-regulation of Wnt signaling in gastric cancer cell line, AGS [J]. Exp Cell Res, 2005,308(1):18-28.

[6] AL-Sarrf M. Treatment of locally advanced head and neck cancer [J]. Cancer Control, 2002,9(5):387-399.

[7] Rubin LL, Philpott KL, Brooks SF. The cell cycle and cell death [J]. Curr Biol, 1993,3(6):391-394.

[8] Gallant P, Nigg E A. Cyclin B2 undergoes cell cycle-dependent nuclear translocation and, when expressed as a non-destructible mutant, causes mitotic arrest in HeLa Cells [J]. J Cell Biol, 1992,117(1):213-224.

[9] Yoshitome S, Furuno N, Hashimoto E, et al. The C-terminal seven amino acids in the cytoplasmic retention signal region of cyclin B2 are required for normal bipolar spindle formation in xenopus oocytes and embryos [J]. Mol Cancer Res, 2003,1(8):589-597.

[10] Abe Y, Takeuchi T, Kagawa-Miki L, et al. A Mitotic kinase TOPK enhances Cdk1/cyclin B1-dependent phosphorylation of PRC1 and promotes cytokinesis [J]. J Mol Biol, 2007,370(2):231-245.

[11] Mollinari C, Kleman JP, Jiang W, et al. PRC1 is a microtubule binding and bundling protein essential to maintain the mitotic spindle midzone [J]. J Cell Biol, 2002,157(7):1175-1186.

[12] Zhu C, Lau E, Schwarzenbacher R, et al. Spatiotemporal control of spindle midzone formation by PRC1 in human cells [J]. Proc Natl Acad Sci U S A, 2006,103(16):6196-6201.

[13] Kishi H, Igawa M, Kikuno N, et al. Expression of the survivin gene in prostate cancer: correlation with clinicopathological characteristics, proliferative activity and apoptosis [J]. J Urol, 2004,171(5):1855-1860.

[14] Beardmore VA, Ahonen LJ, Gorbsky GJ, et al. Survivin dynamics increases at centromeres during G_2/M phase transition and is regulated by microtubule-attachment and Aurora B kinase activity [J]. J Cell Sci, 2004,117(18):4033-4042.

[15] Zhonghong L, Lianjie L, Changqing Z, et al. The influence of survivin shRNA on the cell cycle and the invasion of SW480 cells of colorectal carcinoma [J]. J Exp Clin Cancer Res, 2008,27:20.