Journal of Hainan Medical University

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Experimental study about the regulating effect of Par−4 gene overexpression on the nephroblastoma sensitivity to cisplatin

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ARTICLE INFO

Article history:
Received 2 Nov 2017
Received in revised form 9 Nov 2017
Accepted 12 Nov 2017
Available online 28 Nov 2017

Keywords:
Nephroblastoma
Prostate apoptosis response-4
Cisplatin
Sensitivity

ABSTRACT

Objective: To study the regulating effect of Par-4 gene overexpression on the nephroblastoma sensitivity to cisplatin. **Methods:** Nephroblastoma SK-NEP-1 cells were cultured and divided into four groups, control group were treated with RMPI-1640 without serum or drugs, cisplatin group were treated with serum-free RMPI-1640 containing 5 μg/mL cisplatin, Par-4 group were transfected by Par-4 overexpression plasmids with serum-free RMPI-1640, and cisplatin + Par-4 group were transfected by Par-4 overexpression plasmid with serum-free RMPI-1640 containing 5 μg/mL cisplatin. The cell proliferation activity as well the expression of apoptosis genes, migration genes and invasion genes was measured. **Results:** 8 h, 16 h and 24 h after different conditions of treatment, the cell proliferation activity of cisplatin group, Par-4 group and cisplatin + Par-4 group were significantly lower than that of control group, and the cell proliferation activity of cisplatin + Par-4 group was significantly lower than that of cisplatin group and Par-4 group; 24 h after different conditions of treatment, Bim, PDCD4, WT1, RGS4, Axin, KAI1, E-cadherin, PPAR γ and PTEN mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly higher than those in control group whereas GDNF, GFR α 1, TUBB3, NME1 and FGF1 mRNA expression were greatly lower than those in control group; Bim, PDCD4, WT1, RGS4, Axin, KAI1, E-cadherin, PPAR γ and PTEN mRNA expression in cisplatin + Par-4 group were greatly higher than those in cisplatin group and Par-4 group whereas GDNF, GFR α 1, TUBB3, NME1 and FGF1 mRNA expression were greatly significantly lower than those in cisplatin group and Par-4 group. **Conclusion:** Par-4 gene overexpression can increase the nephroblastoma sensitivity to cisplatin, reduce cell proliferation activity, promote apoptosis and inhibit cell migration and invasion.

1. Introduction

Nephroblastoma is the most common urological malignant tumor in childhood, it is with high mortality, recurrence rate and metastasis rate, and the overall prognosis is not optimistic[1,2]. Cisplatin is the common chemotherapy drug used for nephroblastoma, it has exact killing effects on tumor cells, but the tumor cell resistance to cisplatin during treatment is the important cause of chemotherapy failure[3]. Therefore, how to improve the sensitivity of nephroblastoma cells to cisplatin and avoid the generation of chemotherapy resistance is a clinical problem to be solved at present. Prostate apoptosis response-4 (Par-4) is a new type of pro-apoptotic gene discovered in recent years, which can induce apoptosis through the endoplasmic reticulum stress pathway. Existing animal study has proved that Par-4 can enhance the sensitivity of nephroblastoma animal models to cisplatin[4], but it is not yet clear about the Par-4 effect on the nephroblastoma cell sensitivity to cisplatin, and the downstream genes regulated by Par-4 are also not elucidated. In the following studies, we studied the SK-NEP-1 cells cultured in vitro, and specifically analyzed the regulation effect of Par-4 gene overexpression on the nephroblastoma cell sensitivity of cisplatin.

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Fund Project: Science and Technology Project of Enshi Prefecture No: 160825.

2. Experimental materials and methods

2.1 Experimental materials

Nephroblastoma cells SK-NEP-1 were bought from the cell bank of Chinese Academy of Sciences, the reagents for cell culture and sub-culture were bought from Hyclone Company, cisplatin was purchased from Sigma Company, Par-4 gene overexpression plasmid was synthesis by Shanghai SunBio Biomedical Technology Company, cell viability CCK-8 detection kits were bought from Shanghai Beyotime Company, and RNA expression detection kits were bought from Promega Company.

2.2 Experimental methods

2.2.1 Cell culture and treatment

The SK-NEP-1 cells were cultured with RMPI-1640 containing 10% fetal bovine serum, and the culture medium was replaced once every 3 d until the cell density reached 90%; the cells were digested with trypsin, then collected and inoculated in the culture plate, which were divided into four groups and treated with different conditions: (1) control group were treated with RMPI-1640 without serum or drugs; (2) cisplatin group were treated with serum-free RMPI-1640 containing 5 μg/mL cisplatin; (3) Par-4 group were transfected by Par-4 overexpression plasmids with serum-free RMPI-1640; (4) cisplatin + Par-4 group were transfected by Par-4 overexpression plasmids with serum-free RMPI-1640 containing 5 μg/mL cisplatin.

2.2.2 Cell viability detection

SK-NEP-1 cells were digested and then inoculated in 96-well culture plate, 20 μL CCK-8 detection liquid was added in the culture wells 8 h, 16 h and 24 h after treatment with different conditions, the cells were cultured in the incubator for 4 h, the culture plate was taken out, and the absorbance at 450 nm wavelength was read from the microplate reader and used as the cell proliferation activity value.

2.2.3 Gene expression detection

SK-NEP-1 cells were digested and then inoculated in 6-well culture plate, the culture medium was abandoned 24 h after treatment with different conditions, the kit was used to extract the RNA in cells and synthesize it into cDNA, a suitable amount of cDNA sample was collected for fluorescence quantitative PCR reaction, and the Bim, PDCD4, WT1, RGS4, Axin, GDNF, GFR 1, TUBB3, NME1 and FGF1 mRNA expression were calculated.

2.3 Statistical methods

SPSS 18.0 software was used to input the experimental data, the differences in data among four groups were by variance analysis and $P<0.05$ meant statistical significance in differences in analysis results.

3. Results

3.1 SK-NEP-1 cell proliferation activity

8 h, 16 h and 24 h after treatment with different conditions, analysis of cell proliferation activity among the three groups was as follows: the cell proliferation activity of cisplatin group, Par-4 group and cisplatin + Par-4 group were significantly lower than that of control group ($P<0.05$); the cell proliferation activity of cisplatin + Par-4 group was significantly lower than that of cisplatin group and Par-4 group ($P<0.05$); the cell proliferation activity of cisplatin group was not different from that of Par-4 group ($P>0.05$).

Table 1.

Effect of different conditions of treatment on SK-NEP-1 cell proliferation activity.

Groups	n	8 h	16 h	24 h
Control group	6	0.67±0.09	1.09±0.15	1.44±0.18
Cisplatin group	6	0.44±0.07*	0.69±0.09*	0.84±0.11*
Par-4 group	6	0.48±0.08*	0.74±0.11*	0.89±0.14*
Cisplatin+Par group	6	0.31±0.05*#&	0.48±0.07*#&	0.63±0.09*#&

*: compared with control group, $P<0.05$; #: compared with cisplatin group, $P<0.05$; &: comparedwith Par-4 group, $P<0.05$.

3.2 Pro-apoptosis gene expression in SK-NEP-1 cells

24 h after different conditions of treatment, analysis of pro-apoptosis genes Bim, PDCD4, WT1, RGS4 and Axin expression in three groups of cells was as follows: Bim, PDCD4, WT1, RGS4 and Axin mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly higher than those in control group, and Bim, PDCD4, WT1, RGS4 and Axin mRNA expression in cisplatin + Par-4 group were greatly higher than those in cisplatin group and Par-4 group.

Table 2.

Effect of different conditions of treatment on pro-apoptosis genes in SK-NEP-1 cells.

Groups	n	Bim	PDCD4	WT1	RGS4	Axin
Control group	6	1.03±0.16	1.05±0.11	0.97±0.15	0.99±0.14	1.06±0.18
Cisplatin group	6	1.65±0.22*	1.49±0.19*	1.54±0.18*	1.61±0.19*	1.72±0.20*
Par-4 group	6	1.62±0.24*	1.52±0.18*	1.58±0.17*	1.58±0.18*	1.80±0.26*
Cisplatin+Par group	6	2.94±0.41*#&	3.31±0.49*#&	2.74±0.45*#&	3.06±0.51*#&	3.11±0.49*#&

*: compared with control group, $P<0.05$; #: compared with cisplatin group, $P<0.05$; &: comparedwith Par-4 group, $P<0.05$.

Table 3.

Effect of different conditions of treatment on pro-migration and pro-invasion genes in SK-NEP-1 cells.

Groups	*n*	GDNF	GFR α 1	TUBB3	NME1	FGF1
Control group	6	1.02±0.16	0.96±0.14	1.06±0.18	1.03±0.19	0.98±0.11
Cisplatin group	6	0.66±0.09*	0.73±0.04*	0.59±0.07*	0.68±0.09*	0.70±0.06*
Par-4 group	6	0.69±0.10*	0.76±0.08*	0.62±0.09*	0.65±0.08*	0.64±0.09*
Cisplatin+Par group	6	0.31±0.06*&#	0.37±0.05*&#	0.28±0.04*&#	0.40±0.07*&#	0.34±0.06*&#

*: compared with control group, $P<0.05$; #: compared with cisplatin group, $P<0.05$; &: comparedwith Par-4 group, $P<0.05$.

Table 4.

Effect of different conditions of treatment on anti-migration and anti-invasion genes in SK-NEP-1 cells.

Groups	*n*	KAI1	E-cadherin	PPAR γ	PTEN
Control group	6	1.05±0.18	1.02±0.14	1.06±0.17	0.98±0.11
Cisplatin group	6	1.76±0.23*	1.66±0.21*	1.79±0.24*	1.59±0.19*
Par-4 group	6	1.72±0.26*	1.70±0.24*	1.71±0.21*	1.65±0.22*
Cisplatin+Par group	6	3.19±0.45*#&	2.93±0.33*#&	3.45±0.49*#&	3.27±0.55*#&

*: compared with control group, $P<0.05$; #: compared with cisplatin group, $P<0.05$; &: compared with Par-4 group, $P<0.05$.

3.3 Migration and invasion gene expression in SK-NEP-1 cells

24 h after different conditions of treatment, analysis of pro-migration and pro-invasion genes GDNF, GFR α 1, TUBB3, NME1 and FGF1 expression in three groups of cells was as follows: GDNF, GFR α 1, TUBB3, NME1 and FGF1 mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly lower than those in control group, and GDNF, GFR α 1, TUBB3, NME1 and FGF1 mRNA expression in cisplatin + Par-4 group were greatly significantly lower than those in cisplatin group and Par-4 group.

24 h after different conditions of treatment, analysis of anti-migration and anti-invasion genes KAI1, E-cadherin, PPAR γ and PTEN expression in three groups of cells was as follows: KAI1, E-cadherin, PPAR γ and PTEN mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly higher than those in control group, and KAI1, E-cadherin, PPAR γ and PTEN mRNA expression in cisplatin + Par-4 group were greatly higher than those in cisplatin group and Par-4 group.

4. Discussion

Nephroblastoma is the most common malignant urinary tract tumor in childhood, the radiochemotherapy, surgery and other comprehensive treatments in recent years have been developed continuously and the prognosis of children with nephroblastoma has been improved, but some children still develop tumor recurrence and metastasis due to insensitive chemotherapy, and the prognosis is poor. Cisplatin is a common drug for nephroblastoma chemotherapy, the reduced tumor cell susceptibility to cisplatin may affect the effect of chemotherapy, and the combination of different drugs to improve cisplatin sensitivity is the conventional thinking for clinically improving the curative effect of nephroblastoma. Par-4 is a newly discovered tumor suppressor gene that promotes apoptosis in recent years, the products encoded by the gene can identify the GRP78 on cell membrane surface and start the endoplasmic reticulum stress to induce apoptosis[5,6]. Existing animal study has confirmed that increasing Par-4 expression can improve the sensitivity of nephroblastoma to cisplatin[7]. Analysis of the cell proliferation activity after cisplatin treatment and Par-4 overexpression in the study showed that the cell proliferation activity of cisplatin group, Par-4 group and cisplatin + Par-4 group were significantly lower than that of control group. This indicates that both cisplatin treatment and Par-4 overexpression can reduce the proliferation activity of nephroblastoma. Further analysis of the effect of cisplatin treatment combined with Par-4 overexpression on the cell proliferation activity in the study showed that the cell proliferation activity of cisplatin +Par group was significantly lower than that of cisplatin group and Par-4 group. This indicates that the combination of cisplatin treatment and Par-4 overexpression can be more effective than cisplatin treatment or Par-4 overexpression alone in inhibiting the proliferation of nephroblastoma.

Inducing apoptosis is an important way for cisplatin treatment and Par-4 overexpression to kill nephroblastoma, Bim, PDCD4, WT1, RGS4 and Axin are the currently known pro-apoptosis genes closely related to nephroblastoma apoptosis. Bim is the pro-apoptotic member in the Bcl-2 family, which can antagonize the function of anti-apoptosis molecule Bcl-2 and promote apoptosis[8]; PDCD4 is a newly discovered neoplastic transformation inhibitor in recent years, which can cause cell cycle to arrest and inhibit cell proliferation; WT1 is a specific gene in the pathogenesis of nephroblastoma, and the products encoded by it can identify target DNA, regulate DNA expression and inhibit cell proliferation[9]; RGS4 is a kind of GTP activator protein, which can promote GTP hydrolysis and affect the G protein signaling pathway to promote apoptosis[10]; Axin is the regulating molecule of Wnt signaling pathway, which can form the homodimer and inhibit the transcriptional activity of TCF so as to antagonize the cell proliferation mediated by Wnt signaling pathway[11]. The analysis of the changes in above pro-apoptosis gene expression after cisplatin treatment and Par-4 overexpression in the study showed that Bim, PDCD4, WT1, RGS4 and Axin mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly higher than those in control group. This indicates that both cisplatin treatment and Par-4 overexpression can

increase the expression of pro-apoptosis genes in nephroblastoma. Further analysis of the effect of cisplatin treatment combined with Par-4 overexpression on pro-apoptosis gene expression showed that Bim, PDCD4, WT1, RGS4 and Axin mRNA expression in cisplatin + Par-4 group were greatly higher than those in cisplatin group and Par-4 group. This means that the combination of cisplatin treatment and Par-4 overexpression can be more effective than cisplatin treatment or Par-4 overexpression alone to promote the expression of pro-apoptosis genes and then induce nephroblastoma cell apoptosis.

Tumor cell migration and invasion are the important biological links in nephroblastoma metastasis, and a variety of pro-migration and pro-invasion genes as well as anti-migration and anti-invasion genes are involved in the regulation of the process. GDNF is a member of the TGF-β family, which can promote cell migration and invasion after recognition of GFR 1[12]; TUBB3 is involved in the composition of microtubule in cells, and can regulate the cell movement activity[13]; NME1 is a ribonucleoside diphosphate kinase that plays a role through Akt and MAPK pathway, and it can promote the infiltrative growth of cells; FGF1 is a cytokine widely promoting growth, which can increase the number of new blood vessels and provide a pathway for cell invasion[14]. KAI1, E-cadherin, PPARγ and PTEN are the molecules that inhibit migration and invasion, and they can inhibit the extracellular matrix hydrolysis and enhance the intercellular polarity and adhesion to make the cells anchor in local lesion and inhibit the cell migration and invasion[15,16]. The analysis of the changes in above migration and invasion gene expression after cisplatin treatment and Par-4 overexpression showed that GDNF, GFRα1, TUBB3, NME1 and FGF1 mRNA expression in cisplatin group, Par-4 group and cisplatin + Par-4 group were greatly lower than those in control group whereas KAI1, E-cadherin, PPARγ and PTEN mRNA expression were greatly higher than those in control group. This indicates that both cisplatin treatment and Par-4 overexpression can decrease the expression of pro-migration and pro-invasion genes in nephroblastoma, and increase the expression of anti-migration and anti-invasion genes in nephroblastoma. Further analysis of the effect of cisplatin treatment combined with Par-4 overexpression on migration and invasion gene expression showed that GDNF, GFRα1, TUBB3, NME1 and FGF1 mRNA expression in cisplatin + Par-4 group were greatly significantly lower than those in cisplatin group and Par-4 group whereas KAI1, E-cadherin, PPARγ and PTEN mRNA expression were greatly higher than those in cisplatin group and Par-4 group. This means that the combination of cisplatin treatment and Par-4 overexpression can be more effective than cisplatin treatment or Par-4 overexpression alone in regulating the expression of migration and invasion genes and thereby inhibit the nephroblastoma migration and invasion.

Par-4 gene overexpression can increase nephroblastoma sensitivity to cisplatin, and the combination of cisplatin treatment and Par-4 overexpression can reduce the cell proliferation activity, increase the expression of pro-apoptosis, anti-migration and anti-invasion genes and decrease the expression of pro-migration and pro-invasion genes so as to promote cell apoptosis and inhibit cell migration and invasion.

References

[1] Ikeda H, Nakamura Y. Trends in incidence of childhood malignant solid tumors in Japan: Estimation based on hospital-based registration. *J Pediatr Surg* 2015; **50**(9): 1506-1512.

[2] Hontecillas-Prieto L, Garcia-Dominguez DJ, Vaca DP, Garcia-Mejias R, Marcilla D, Ramirez-Villar GL, et al. Multidrug resistance transporter profile reveals MDR3 as a marker for stratification of blastemal Wilms tumour patients. *Oncotarget* 2017; **8**(7): 11173-11186.

[3] Wincewicz A, Kowalik A, Zieba S, Kopczynski J, Gozdz S, Sulkowski S. Review of prognostic and predictive aspects of mutated TP53 in Wilms' tumor biology with morphological report and molecular analysis of 37-year-old man's nephroblastoma. *Pol J Pathol* 2016; **67**(4): 307-312.

[4] Wang J, Li Y, Ma F, Zhou H, Ding R, Lu B, et al. Inhibitory effect of Par-4 combined with cisplatin on human Wilms' tumor cells. *Tumour Biol* 2017; **39**(7): 1010428317716689.

[5] Tiruttani Subhramanyam UK, Kubicek J, Eidhoff UB, Labahn J. Structural basis for the regulatory interactions of proapoptotic Par-4. *Cell Death Differ* 2017; **24**(9): 1540-1547.

[6] Burikhanov R, Hebbar N, Noothi SK, Shukla N, Sledziona J, Araujo N, et al. Chloroquine-inducible par-4 secretion is essential for tumor cell apoptosis and inhibition of metastasis. *Cell Rep* 2017; **18**(2): 508-519.

[7] Shen Z, Qin X, Yan M, Li R, Chen G, Zhang J, et al. Cancer-associated fibroblasts promote cancer cell growth through a miR-7-RASSF2-Par-4 axis in the tumor microenvironment. *Oncotarget* 2017; **8**(1): 1290-1303.

[8] Rah B, ur Rasool R, Nayak D, Yousuf SK, Mukherjee D, Kumar LD, et al. PAWR-mediated suppression of BCL2 promotes switching of 3-azido withaferin A (3-AWA)-induced autophagy to apoptosis in prostate cancer cells. *Autophagy* 2015; **11**(2): 314-331.

[9] Nagasaki J, Aoyama Y, Hino M, Ido K, Ichihara H, Manabe M, et al. Wilms tumor 1 (WT1) mRNA expression level at diagnosis is a significant prognostic marker in elderly patients with myelodysplastic syndrome. *Acta Haematol* 2017; **137**(1): 32-39.

[10]LIU Yan-chun, LIU Shi-xia, WANG Xiao-jing, WANG Lian-fang, YANG Ji-hong. Clinical significance of expression of RGS4 in pediatric nephroblastoma tissue. *Tianjin Med J* 2017; **45**(1): 36-39.

[11]Brauburger K, Akyildiz S, Ruppert JG, Graeb M, Bernkopf DB, Hadjihannas MV, et al. Adenomatous polyposis coli (APC) membrane recruitment 3, a member of the APC membrane recruitment family of APC-binding proteins, is a positive regulator of Wnt-β-catenin signalling. *FEBS J* 2014; **281**(3): 787-801.

[12]Rickert U, Grampp S, Wilms H, Spreu J, Knerlich-Lukoschus F, Held-Feindt J, et al. Glial cell line-derived neurotrophic factor family members reduce microglial activation via inhibiting p38mapks-mediated inflammatory responses. *J Neurodegener Dis* 2014; **2014**: 369468.

[13]PAN Wei-kang, YU Hui, WANG Huai-jie, ZHENG Bai-jun, GUO Qing-qing, GUO Zheng-tuan, et al. Expression and clinical significance of ERCC1, TUBB3 and TOP2A mRNA in nephroblastoma. *J Xi'an Jiaotong Univ* 2016; **37**(5): 689-692.

[14]Motamedi FJ, Badro DA, Clarkson M, Lecca MR, Bradford ST, Buske FA, et al. WT1 controls antagonistic FGF and BMP-pSMAD pathways in early renal progenitors. *Nat Commun* 2014; **17**(5): 4444.

[15]Balasubramaniam SL, Gopalakrishnapillai A, Petrelli NJ, Barwe SP. Knockdown of sodium-calcium exchanger 1 induces epithelial-to-mesenchymal transition in kidney epithelial cells. *J Biol Chem* 2017; **292**(27): 11388-11399.

[16]Cui M, Liu W, Zhang L, Guo F, Liu Y, Chen F, et al. Over-expression of mir-21 and lower pten levels in wilms' tumor with aggressive behavior. *Tohoku J Exp Med* 2017; **242**(1): 43-52.