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ORIGINAL ARTICLE

Clinical application of patient derived xenograft mouse model in children with neuroblastoma

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Summary

Purpose: *To establish a neuroblastoma patient derived xenograft (PDX) mouse model to select antitumor drug and guide individualized therapy.*

Methods: *The tumor tissues from a child with neuroblastoma were transplanted into NSG mice to establish neuroblastoma PDX. The mice were divided into different groups treated with different anti-tumor drugs for 1 week. The physical condition, weight of mice, the pathological changes and protein expressions were observed to select the best clinical treatment. After individualized therapy, the therapeutic effect was evaluated by glioblastoma specific indicators and imaging examination. The feasibility and effectiveness of the individualized therapy were also verified in another 3 patients.*

Results: *The PDX mouse model was successfully established with the same pathologic characteristics as the child's neuroblastoma. The chemotherapy regimens in group B and D showed toxicity with the larger decrease of weight in mice. The chemotherapy regimen in group G had strong killing ability on tumor with neuroblastoma cell rate of 0%, and negative expression of CD56, CGA, SYN, which was recommended as the optimal regimen. The child completed chemotherapy successfully, and is currently disease-free. During 23-month follow-up, neuron specific enolase was in normal range and no obvious sign of recurrence was found. The individualized therapy based on PDX in another 3 children also obtained good therapeutic effect and prognosis.*

Conclusion: *The neuroblastoma PDX mouse model has been successfully established for drug efficacy evaluation, and the screened regimen can be applied to individualized treatment with favourable prognosis.*

Key words: *neuroblastoma, patient derived xenograft model, individualized therapy, preclinical model*

Introduction

Neuroblastoma is one of the most common malignant solid tumors in children, mainly occurring in the adrenal medulla and paravertebral sympathetic nervous system [1]. The clinical symptoms of neuroblastoma in children are non-specific accounting for about 40%, and the metastatic sites are mostly in the liver, bone, marrow and lymph nodes [2]. Although there has been some progress in the diagnosis and treatment of neuroblastoma in recent years, the mortality rate of high-risk neuroblastoma is still high, and the survival rate in high-risk population in China is still less than 30%. Neuroblastoma is still one of the most major malignant solid tumors threatening the life and health of children [3,4]. Therefore, due to its high morbidity and mortality, it is urgent to find treatment approaches to improve the overall survival of such patients [5,6].

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The development of new antitumor drugs is an important topic for oncology researchers and clinicians, and its key research basis is to establish a stable and effective tumor animal model [7]. Cell line-derived xenograft (CDX) is a traditional tumor model. Briefly, human tumor cells are cultured *in vitro* to establish stable cell lines and these cells are then injected into immunodeficient mice to obtain tumor animal model [3,4]. CDX has always been an effective tool for the clinical efficacy prediction and toxicity evaluation of anti-tumor drugs, which is widely used for its advantages of easy construction, high tumor formation rate and short cycle. However, the drugs screened by an NCI-60Panel anti-tumor drug screening system based on CDX are not appropriate for the clinical treatment of patients, and about 88% of the screened drugs haven't been clinically validated as previously reported [9]. Although CDX have been useful for the identification and testing of many classical cytotoxic drugs, it lacks the tumor heterogeneity hindering the development of anti-tumor drugs [10,11]. Patient-derived xenograft (PDX) is a tumor animal model using highly immunodeficient mice as the carrier, which is constructed by directly transplanting fresh tumor tissue from patient into mice. PDX can well retain the tumor structure and matrix components, and maintain the characteristics of the primary tumor after multiple passages, possessing unique advantages in simulating the growth, immunosuppression and metastasis of tumor tissue [12]. Moreover, PDX can accurately reflect the heterogeneity, phenotype and molecular characteristics of tumor, including gene expression pattern, DNA methylation, mutation and proteomics [12,13]. Meanwhile, PDX also maintains the activity of most key genes and signaling pathways in the primary tumor. Therefore, PDX restores the histopathologic characteristics, growth status and tumor microenvironment of the primary tumor, and plays an important role in the development of tumor markers and new drugs [14]. Based on the above advantages of PDX, the immunodeficient mice can be a reliable alternative to patients for conduction of drug effectiveness and toxicity tests to maximize the therapeutic effect and reduce the toxic effect, and the consistency rate of the medical results by PDX and clinical chemotherapy is as high as 80% [13].

Therefore, our study applied different chemotherapy regimens to neuroblastoma PDX mouse model to conduct drug sensitivity test, and the optimal chemotherapy plan was screened out by combination evaluation of the weight of mouse, tumor Ki-67 value and immunohistochemistry of mice and the characteristics of the patient. The therapeutic effect on patients was also evaluated to verify the feasibility and effectiveness of the chemotherapy plan. With this research, an individualized therapy of children with neuroblastoma based on PDX was established to maximize the therapeutic efficacy and minimize side effect, hoping to provide guidance for clinical treatment.

Methods

Participants and tumor tissues collection

This study was approved by the Ethics Committee and Review Board of the People's Hospital of Hunan Province (No.2), which was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. A 20-month-old female child wa s enrolled in the department of pediatric hematology in our hospital on March 22, 2019, and diagnosed as right adrenal neuroblastoma by pathological examination. Based on the international neuroblastoma staging system (INSS) and Clinical risk by Children's Oncology Group (COG), the neuroblastoma of the child was identified as stage IV and high-risk. The chemotherapy plan, as shown in Table 1, was applied to the child. After multidisciplinary consultation proved that the child was eligible for surgery, the radical resection of neuroblastoma was performed under general anesthesia on July 19, 2019.

The tumor tissues derived from the child with neuroblastoma were harvested in tumor resection. The resected fresh neuroblastoma tumor tissues were placed in Roswell Park Memorial Institute 1640 medium (RPMI1640, Procell, Wuhan, China) along with 0.5% streptomycin, 0.5% penicillin and 20% fetal bovine serum (FBS, Sinopharm Chemical Reagent, Shanghai, China) within 10 min. These tumor tissues were prepared for the establishment of PDX, hematoxylin and eosin (H&E) staining and immunohistochemistry.

Table 1. Chemotherapy plan before the PDX establishment

Therapy period	*Data*	*Chemotherapy plan*
1	2019-04-03	Irinotecan+cyclophosphamide+vincristine
2	2019-04-22	Temozolomide+irinotecan +vincristine
3	2019-05-14	Cisplatin+etoposide
4	2019-06-05	Cyclophosphamide+pirarubicin+vincristine+mesna
5	2019-06-27	Cisplatin+etoposide

PDX establishment

The neuroblastoma tissues were continuously rinsed with a solution of 0.5% streptomycin and penicillin, and their necrotic material and adipose tissue were carefully removed. The tumor tissues of good activity were selected according to the color, structure and toughness, and microscopically cut into tissue fragments of 2×2×2 mm with ophthalmic tweezers and scalpel under sterile environment. The tissue fragments were prepared for animal inoculation, and the process from tissue collection to inoculation should be done within 24 h so as not to affect the activity of tumor cells.

Female NSG mice (18-22 g) aged 5-8 weeks, were purchased from Nanjing Puenrui Biological Technology Co., Ltd. (Nanjing, China), and kept under particular pathogen-free conditions. These mice were fixed on retainer and conditioned by removing hair from the right rear leg. The prepared tumor tissue fragments were put into a membrane carrier and collected by inoculating needle. After skin disinfection, the needle was inserted diagonally along the edge of the skin and then vertically into the muscle tissue. Through this method, the tumor tissues were transplanted into the right rear leg of NSG mice to establish the neuroblastoma PDX. These mice were provided with standard food and water ad libitum, and reared under a constant 12 h / 12 h light/dark regimen at 20-26°C and relative humidity of 40-70%.

Chemosensitivity testing

Chemotherapy regimens and doses were selected by clinicians based on the clinical symptoms, general examination results, imaging data and pathologic examination, as well as tumor stages and types. After transplantation for 4 days, these mice were kept in standard cages with 3 in each, and divided into the control group and the experimental group according to clinical recommended treatment options of each patient (n=3). Group A was the control group without treatment, group B was intraperitoneally injected with vincristine 1.5 mg/m^2 qw, cyclophosphamide 1.8 g/m^2 biw and doxorubicin 25 mg/m^2 tiw. Group C was intraperitoneally injected with vincristine 1.5 mg/m^2 qw, cyclophosphamide 1.8 g/m^2 biw, cisplatin 90 mg/m^2 qiw and etoposide 200 mg/m^2 tiw. Group D was intraperitoneally injected with cisplatin 90 mg/m^2 qiw and etoposide of 200 mg/m^2 tiw, the group E was intraperitoneally injected with ifosfamide 1.5 g/m^2 piw, carboplatin 450 mg/m^2 qw and etoposide 200 mg/m^2 tiw. Group F was treated with irinotecan 50 mg/m^2 by intraperitoneal injection piw, temozolomide 100 mg/ m^2 by oral administration piw, dasatinib 250 mg/m^2 and rapamycin 1 mg/m^2 by oral administration qd, the group G was treated with irinotecan 50 mg/m^2 by intraperitoneal injection piw, temozolomide 100 mg/m^2 by oral administration piw, bevacizumab 15mg/kg and vincristine 1.5 mg/m^2 by intraperitoneal injection piw. These drugs were bought from the Sinopharm Chemical Reagent (Shanghai, China).

During chemotherapy for 1 week, the physical condition and death of mice were observed and recorded every day. In addition, the weight of mice was monitored 0, 3, 7 days after chemotherapy, and their weight changes were calculated with formula: weight changes= (m_7-m_0)/m_0×100%, m_7 was the mice weight at 7 days after chemotherapy, m_0 was the mice weight before chemotherapy (0 days). After the chemosensitivity testing, the mice were euthanized and their tumor tissues were surgically removed.

H & E staining

H&E staining was used for tumor morphology, proportion of tumor components, neuroblastoma cell rate and tumor cell necrosis rate (TCNR) analysis. The neuroblastoma tissues were fixed with 10% formalin solution (Sinopharm Chemical Reagent, Shanghai, China) for 48 h, and embedded in paraffin after dehydration. Sections (4 μm) were cut from the prepared wax blocks by the RM2235 Bio-tissue embedding machine (Leica, Wetzlar, Germany). H&E staining was carried out following the manufacturer's protocol for the G1120 H&E staining Kit (Solarbio, Beijing, China). The morphological structures of neuroblastoma tissues were observed under DMi8 microscope (Leica, Wetzlar, Germany), and tumor heteromorphism, tumor cell rate and TCNR were analyzed by the pathology experts.

Immunohistochemistry

The expression of Ki-67, CD56, chromogranin A (CGA) and synaptophysin (SYN) was evaluated by immunohistochemistry, which was carried out by the Immunohistochemical kit (Jiangsu Kangwei Shiji, Taizhou, China). After slicing, sections (4 μm) were baked at 60°C for 2 h, and deparaffinized in xylene and rehydrated in decreasing alcohol concentrations. The sections of antigen retrieval were boiled in pH 6 citrate buffer (Boster, Wuhan, China) for 15 min, and then blocked with 3% H_2O_2 for 20 min at room temperature for endogenous peroxidase neutralization. After incubation in 10% bovine serum albumin (Boster, Wuhan, China) blocking solution at 37°C for 30 min, sections were incubated with the rabbit monoclonal anti-Ki-67 (1:200, ab16667, Abcam, Cambridge, MA, USA), anti-CD56 (1:200, 99746T, Cell Signaling Technology, Danvers, MA, USA), anti-CGA (1:100, SP12, Maxim, China) and anti-SYN (1:100, SP11, Maxim, China) overnight at 4°C. The antigen-antibody reactions were detected with secondary antibody conjugated with hyperoxide peroxidase (HRP), visualized with diaminobenzidine (DAB). Stained sections were observed under DMi8 microscope (Leica, Wetzlar, Germany). The Ki-67 index was calculated as the percentage of positive cells per 1,000 counts of the total cells [15].

Individualized treatment and prognostic evaluation

The screened optimal chemotherapy plan by PDX was applied to the child every three weeks. When the efficacy of chemotherapy regimens in different groups based PDX was comparable, the regimens could be used alternately. The neuroblastoma markers including neuron specific enolase (NSE) and 24 h urine vanilla mandelic acid (VMA), as well as regular imaging examination were reviewed during chemotherapy.

Clinical application

Another 3 children with neuroblastoma were enrolled from August 1, 2019 to February 1, 2021, and accepted the individualized therapy based on PDX as previously described. The therapeutic effect was evaluated to verify the feasibility and effectiveness of the individualized therapy. All of these children including the aforementioned one and their parents understood and agreed with the purpose of our study. Written informed consent was obtained from all of them.

Statistics

SPSS 22.0 software (SPSS Inc., IBM, Armonk, NY, USA) was used for statistical analyses. Data were expressed as mean values ± standard deviation. Differences among groups were analyzed by one-way analysis of variance (ANOVA) and the pairwise comparisons of data were analyzed by Least Significant Difference (LSD) test. $P < 0.05$ was considered as significant.

Results

Identification of PDX mouse model

After the transplantation of neuroblastoma tissues, the mice were able to move freely and feed by themselves, no blood and fluid oozing in the surgical wound, no obvious infection, no death was observed. Before chemotherapy, the neuroblastoma tissues of mice and patient were identified by H&E staining and immunohistochemistry, as shown in Figure 1. In PDX mouse model, the tumor cell was circular or ovoid, showing invasive growth, mild atypia, diffuse distribution, and the cell boundary was not clear. Moreover, the nuclei were small and round, chromatin was coarsely granular, pathologic fission occurred, cytoplasm was empty, and a small amount of necrosis in the stroma could be observed, which was similar to the H&E staining of the primary tumor in the child with neuroblastoma (Figure 1A). In addition, immunohistochemistry showed that the expressions of CD56, CGA and SYN were positive in both PDX mouse model and the child with neuroblastoma (Figure 1B-D). Hence, it was suggested that the PDX mouse model was successfully established with the same pathologic characteristics as the child's neuroblastoma.

Weight changes of mice during chemotherapy

During chemotherapy there was no obvious abnormal activity, infection and death. The weight of mice in each group was monitored 0, 3 and 7 days, and shown in Table 2. The weight of mice in the group B decreased by about 20% within 1 week, indicating the high toxicity of the chemotherapy regimen. The weight of mice in group D decreased

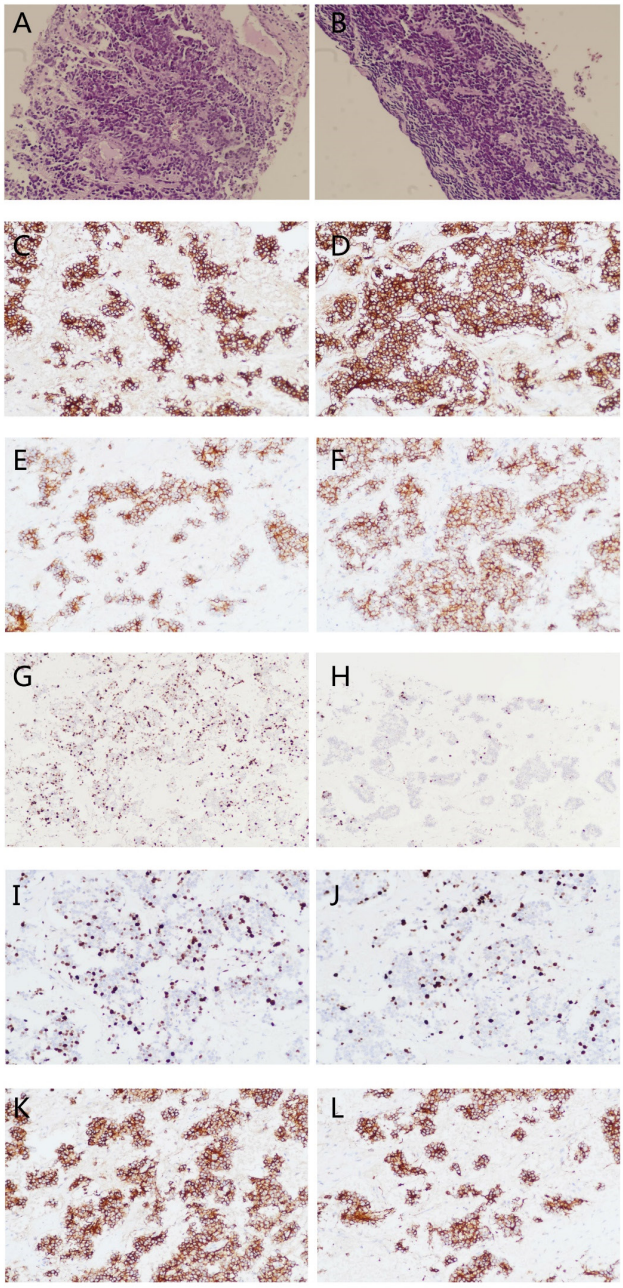

Figure 1. Pathological characteristics and protein expressions of neuroblastoma tissues in the child and PDX mouse model. **A:** neuroblastoma tissues in the child by H&E staining with magnification of ×20. **B:** neuroblastoma tissues in the PDX mouse model by H&E staining with magnification of ×20. **C:** CD56 expressions in the child by immunohistochemistry with magnification of ×20. **D:** CD56 expressions in the PDX mouse model by immunohistochemistry with magnification of ×20. **E:** CGA expressions in the child by immunohistochemistry with magnification of ×20. **F:** CGA expressions in the PDX mouse model by immunohistochemistry with magnification of ×20. **G:** Ki-67 expressions in the child by immunohistochemistry with magnification of ×20. **H:** Ki-67 expressions in the PDX mouse model by immunohistochemistry with magnification of ×20. **I:** SYN expressions in the child by immunohistochemistry with magnification of ×20. **J:** SYN expressions in the PDX mouse model by immunohistochemistry with magnification of ×20.

Table 2. The weight changes of mice during chemotherapy (n=3, g)

Group	*Chemotherapy regimens*	*Time after chemotherapy (d)*			*Weight changes (%)*
		0	*3*	*7*	
Group A	None	18.87±0.92	19.45±1.18	19.67±1.07	4.24
Group B	Vincristine+cyclophosphamide+doxorubicin	19.51±0.97	17.8±0.99	15.46±0.84	-20.76
Group C	Vincristine+cyclophosphamide+cisplatin+etoposide	20.40±1.06	20.16±1.10	18.42±1.01	-9.71
Group D	Cisplatin+ etoposide	20.48±1.05	19.44±1.08	17.39±0.90	-15.09
Group E	Ifosfamide+carboplatin+ etoposide	18.29±1.01	17.04±0.87	16.72±0.82	-8.58
Group F	Irinotecan+temozolomide+dasatinib+rapamycin	18.46±0.96	17.83±0.94	17.44±0.88	-5.53
Group G	Irinotecan+temozolomide+bevacizumab+vincristine	20.64±1.15	20.12±1.04	18.75±0.95	-9.16

Table 3. Pathologic characteristics and protein expression in different groups (n=3)

Group	*Heteromorphism*	*Proportion of tumor components (%)*	*Neuroblastoma cell rate*	*TCNR*	*Ki-67 index (%)*	*Expression of CD56*	*Expression of CGA*	*Expression of SYN*
Group A	Mild	60	5	None	<5	+++	+++	+++
Group B	Mild	90	20	None	<5	+++	+++	+++
Group C	Mild	80	40	None	<5	+++	+++	+++
Group D	Mild	60	0	None	<5	-	-	-
Group E	Mild	70	10	None	<5	+++	+++	+++
Group F	Mild	50	10	None	<5	+++	+++	+++
Group G	Mild	20	0	None	<5	-	-	-

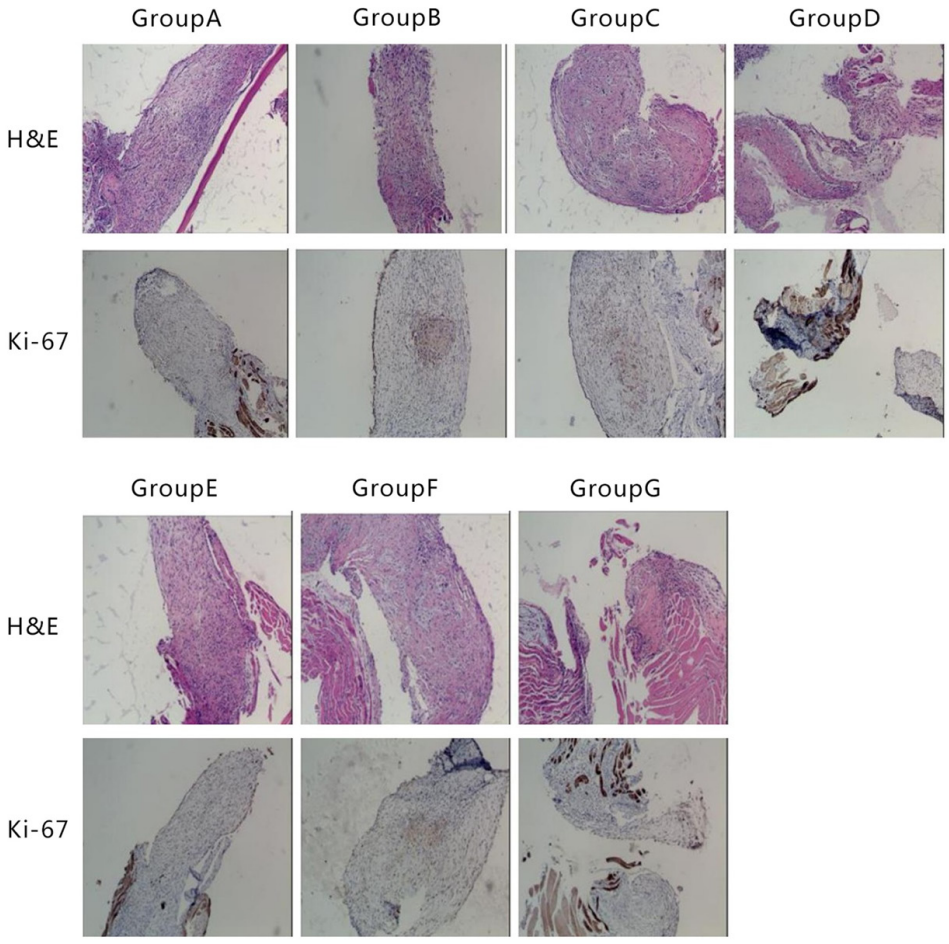

Figure 2. Pathologic characteristics and Ki-67 expressions of neuroblastoma tissues in different groups by H&E staining and immunohistochemistry. The magnification of all pictures is ×20.

at least 10%, also indicating the drug toxicity. The weight of mice in other groups changed steadily, and no weight change was significantly greater than 10%, indicating that the chemotherapy regimens in other groups had good safety.

Pathologic characteristics and protein expression

H&E staining was used for tumor morphology, proportion of tumor components, neuroblastoma cell rate and tumor cell necrosis rate (TCNR) analysis, and immunohistochemistry was performed to evaluate the Ki-67 index and expression of CD56, CGA and SYN (Table 3 and Figure 2). The heteromorphism of neuroblastoma was mild, the Ki-67 index was less than 5%, and no TCNR was observed in all groups. However, the proportion of tumor components was 60%, neuroblastoma cell rate was 0%, the expression of CD56, CGA and SYN was negative in group D (cisplatin+ etoposide), and these indexes were 20%, 0% and negative in group G (irinotecan+ temozolomide+ bevacizumab+ vincristine), respectively. These results demonstrated that the chemotherapy regimens in group D and G have strong killing ability on tumor. Therefore, the chemotherapy regimen of group G could be recommended as the optimal regimen for the child with neuroblastoma by PDX.

Individualized treatment and prognosis

The screened optimal chemotherapy plan by PDX (irinotecan+ temozolomide+ bevacizumab+ vincristine), was applied to the child in August 26,

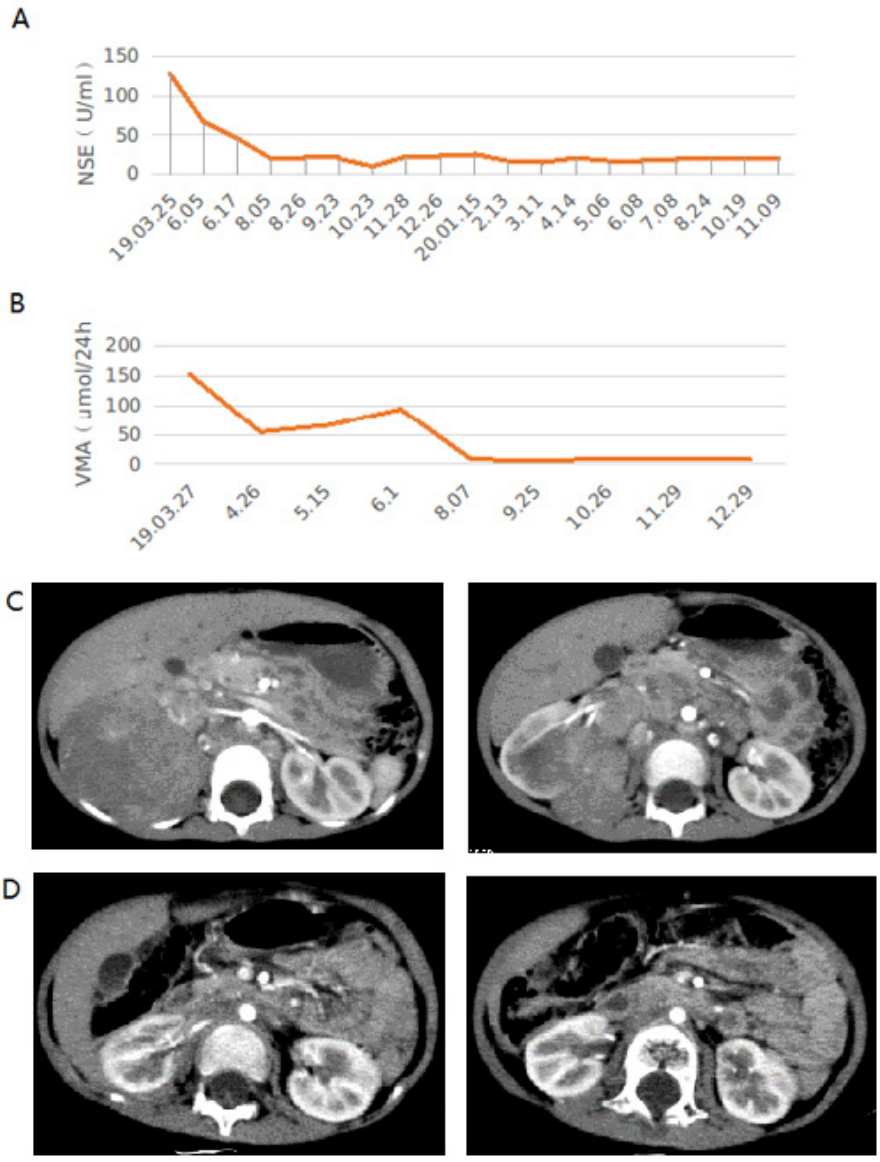

Figure 3. Prognosis results of the child with neuroblastoma by NSE, VMA levels and imaging examination. **A:** NSE level in different time points of follow-up. **B:** VMA level in different time points of follow-up. **C:** imaging examination before the PDX establishment by the abdominal CT. **D:** imaging examination during follow-up by abdominal CT.

Table 4. Information and treatment of another 3 children with neuroblastoma

Patient number	*Gender*	*Age (month)*	*Diagnosis*	*Location of tumor*	*INSS/COG*	*Chemotherapy regimen by PDX*	*Follow-up time (months)*	*Prognosis*
1	Female	6	Neuroblastoma	mediastinum	Stage II/ low-risk	Irinotecan + temozolomide + vincristine	5	complete remission with normal NSE
2	Male	16	Neuroblastoma	retroperitoneum	Stage III/ high-risk	Irinotecan + temozolomide + dasatinib + rapamycin	11	complete remission with normal NSE
3	Female	25	Neuroblastoma	right adrenal gland	Stage IV/ high-risk	Cis-platinum+ etoposide	6	complete remission with normal NSE

2019, which was used for a therapy period of about 3 weeks. The child completed chemotherapy successfully on January 23, 2020, and her neuroblastoma markers NSE and VMA, as well as imaging examinations were checked regularly.

As indicated in Figure 3, the levels of VMA and NSE in the child were obviously higher in the early diagnosis and treatment, and began to decline significantly after chemotherapy. Although the level of VMA revealed a trend of fluctuations during chemotherapy, it did not fluctuate after decline to normal. In addition, the preserve of urine for 24 h in the later follow-up required more attention and time, so the NSE was the main examination index in the follow-up. There was still no significant fluctuation of NSE after decline to normal. As for the abdominal CT image after chemotherapy for 6 months (Figure 3), it suggested postoperative changes in the right adrenal. No obvious new abnormal density and enhancement area was observed in retroperitoneum, multiple lymph nodes were observed, but none of them was obviously enlarged. Mild hydronephrosis of bilateral renal pelvis was improved compared with before. The child had complete remission evaluated by oncologists in our hospital after 23 months, indicating the chemotherapy plans by PDX was consistent with the clinical condition of the child, and obtain a good therapeutic effect on neuroblastoma. Therefore, it is feasible to apply PDX on antitumor drug selection and individualized therapy.

Clinical application on 3 children

In consideration of the successful PDX application, another 3 children with neuroblastoma accepted the individualized therapy based on PDX, and their information as well as treatment is indicated in Table 4. Although the information including gender, age, location and grading of tumor were different, their individualized therapy based on PDX could obtain good therapeutic effect and prognosis, which revealed the feasibility and effectiveness of the individualized therapy in clinical practice.

Discussion

Neuroblastoma's incidence in children accounts for about 8-10%, and most neuroblastomas are diagnozed with systemic metastases and rapidly become terminal stages with very poor prognosis [16,17]. As reported, 51 neuroblastoma patients who survived for at least one year after comprehensive treatment including intensive chemotherapy, radiotherapy and surgery, were followed up, revealing the height of most patients was significantly influenced, their hearing and teeth were also affected to some extent, 50% of high-risk neuroblastoma survivors developed early diabetes or diabetes, 59% had thyroid insufficiency, and 75% had ovarian insufficiency [18].

PDX is a preclinical model with predictive value, which is able to maintain the primary tumor properties and tumor stroma in mice [19]. In our study, tumor fragments rather than tumor cells were transplanted into mice, which ensured the structure of tumor after implantation, and better restored the living environment of the primary tumor cells as well as the most active pathways. Based on the pathologic characteristics and protein expression, the PDX mouse model was successfully established with pathologic characteristics as the child's neuroblastoma.

At present, neuroblastoma patients are given chemotherapy according to their groups, however, high tumor heterogeneity exists between different patients in the same group. PDX can reflect the differences between individuals, which is more true and pertinent to guide clinical medication [14].

The success rate of PDX establishment is positively correlated with the tumor malignant grade. The lower the differentiation grade of primary tumor, the higher the tumor malignant grade, and the higher the success rate of PDX establishment [20]. Since 70-80% of children in China are in terminal stages when diagnosed with neuroblastoma [21], the success rate of neuroblastoma PDX establishment was relatively high, and the PDX mouse model was successfully established in the first child and the other 3 children with neuroblastoma in our study. At present, PDX has been applied in adult preclinical research and individualized treatment of lung cancer, breast cancer and other malignant tumors, which has achieved good effect. For example, about one-third of non-small cell lung cancer (NSCLC) in adults develop resistance, PDX has become a common indicator for evaluating the efficacy of adult NSCLC before chemotherapy, and has guiding significance for patients' medication [22]. Meanwhile, PDX still holds great promise for children application. As reported, the genome-wide chromatin and epigenetic characters of T-cell acute lymphoblastic leukemia in children can be retained in PDX [23].

In the study of Cramer et al, mutations in BCOR, ARID1A and SETD2 genes, contributed to epigenetic regulation and interacted with or modified the activity of histone deacetylases (HDAC) in a 8-year-old child with rhabdomyosarcoma. The antitumor activity of HDAC inhibitor vorinostat observed with the PDX model developed from this patient's tumor, reflected the efficacy of this agent in clinical observations [24]. Likewise, the neuroblastoma PDX mouse model was established to screen the optimal chemotherapy plan for individualized therapy in our study. The greater the weight loss, the greater the toxicity of the drug. According to the results of physical condition and weight changes, the chemotherapy regimens including vincristine+cyclophosphamide+doxorubic in and cisplatin+ etoposide, were excluded because of high toxicity. The proportions of tumor components in all groups were more than 5%, which suggested the reliability of results on tumor in mice. The higher the TCNR, the greater the lethality of the anti-tumor drug to tumor, indicating good efficacy of chemotherapy regimen.

The Ki-67 index can reflect the tumor cell proliferation, and its decrease indicates that the chemotherapy regimen has an inhibitory effect on the tumor cell proliferation. However, TCNR and Ki-67 index aren't able to select the optimal chemotherapy regimen for several reasons, for instance, the patient has undergone multiple chemotherapy before PDX establishment, leading to significant decline of TCNR and Ki-67 index. As shown in this study, the chemotherapy plan has been applied to the child, and the Ki-67 index was less than 5%, and no TCNR was observed in all groups with PDX. Hence, the assisting determination, such as neuroblastoma cell rate, has been observed in our study. The higher the neuroblastoma cell rate, the worse the therapeutic effect of chemotherapy regimen. The neuroblastoma cell rate was 0%, and the expression of CD56, CGA and SYN was negative in group D and G, so these chemotherapy regimens have strong killing ability on tumor. Combined with the results of drug toxicity, the chemotherapy regimen of group G can be recommended as the optimal regimen for the child with neuroblastoma. After application of the optimal chemotherapy plan, that is irinotecan+ temozolomide+ bevacizumab+ vincristine, the child completed chemotherapy successfully, and is currently surviving disease-free. During the 23-month follow-up, NSE, as a specificity index of neuroblastoma, has been in normal range after chemotherapy for 1 year, and no obvious signs of recurrence have been found by image examination in the child. The feasibility and effectiveness of the individualized therapy were verified in another 3 children with neuroblastoma, and their individualized therapy based on PDX could obtain good therapeutic effect and prognosis. The success of an individualized therapy based on neuroblastoma PDX in our study, got better preliminary results in clinical practice. The individualized therapy not only can be used for adult malignancies [25,26], but also applied to solid tumor in children, especially children in high-risk and recurrent solid tumor, which can reduce the adverse reactions, as well as the inhibition of growth and development caused by unnecessary intense chemotherapy.

Compared to the traditional CDX, PDX preserves not only the primary tumor cells but also the primary tumor stroma. It has been shown in the literature that tumor cells cultured in PDX model can basically keep the same character as the original tumor within 5 generations [20,27]. Thus, PDX is better to get close to the primary tumor and improve the effectiveness of new drug researches and personalized therapies. There is no application of PDX on neuroblastoma through literature review [28,29], but the neuroblastoma PDX mouse model has been successfully established in our study for the first time, which has been used for drug efficacy

evaluation to guide individualized therapy with favourable prognosis.

In this study, there are some shortcomings. The degree of activity and freshness of selected tumor tissues can directly affect the success rate of PDX establishment. On the other hand, the establishment of PDX model requires a certain cost due to the high price of NSG mice. The subjects in this study are the children with neuroblastoma, who are willing to accept the individualized therapy based on PDX and it is impossible to obtain a larger sample size only in our hospital. Cooperation from multiple teams and organizations is necessary to obtain a large number of sample data in the future studies. Expansion of study subjects for PDX can also provide help for the etiology, pathogenesis and therapeutic method of children with neuroblastoma. Moreover, the individualized therapy based on PDX may also be applied to other high-risk and refractory pediatric solid tumors, such as hepatoblastoma, nephroblastoma, soft tissue sarcoma, and so on.

Conclusion

To our knowledge, this is the first description of the clinical application of PDX mouse model in children with neuroblastoma. In our study, the neuroblastoma PDX mouse model has been successfully established for drug efficacy evaluation, and the screened regimen can be applied to individualized treatment with favourable prognosis. Our study has provided evidence for the feasibility and effectiveness of the individualized therapy based on PDX to provide the foundation for neuroblastoma therapy and future studies.

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Conflict of interests

The authors declare no conflict of interests.

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