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Neuroblastoma Treatment Using Gene Therapy

A. Anusha[1], Harshit Palecha[2]

[1,2]AMITY Institute of Biotechnology, AMITY University Rajasthan, Jaipur, India
Email – [1]anumagical@gmail.com, [2]harpalecha@gmail.com

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Abstract: **Neuroblastoma is a type of pediatric malignancy, characterised by high clinical heterogeneity. It starts from primitive nerve cells found in areas where nerves join together. The tumor regresses spontaneously in infants and on maturation differentiates into benign ganglioneuroblastoma in later stages. The tumor is peripheral and generally present on the sympathethic nervous system. In most of the solid tumors, tumor initiating cells (TICs) are present, which helps in fuelling the growth of tumor and start the metastases. Neuroblastoma typically spreads to the regional lymph nodes, bone and bone marrow. The mean age of diagnosis is 2 years, almost 1/4th of the cases occur before the age of 1 year and the remaining occur before 10 years of age. Symptoms generally depend on the size, location of the original tumor and extent to spread to the other parts of the body. Some prominent symptoms are pain, high blood pressure, problem in the movement of lower limb. Mostly in children it is recognized by the presence of an unusual lump in any part of the body (mainly abdominal region). Presence of common DNA variations in a region of chromosome 6 raises the risk of aggressive form of neuroblastoma in the child. Further sequencing of the chromosome region identified mutations in the anaplastic lymphoma kinase (ALK) gene in families affected by neuroblastoma. The epigenetic structure of DNA and its lesions play a role in the origin of human neuroblastomas due to which other treatment options for neuroblastomas can be created by the pharmaceutical manipulation of the epigenome. Cold atmospheric plasma (CAP) can be used as a treatment option for neuroblastoma since apoptosis can be induced in neuroblastoma cells using CAP. It also inhibits the metabolic rate of neuroblastoma cells.**

Some treatment techniques for high risk neuroblastoma are immunotherapy, Myeloablative therapy, chemotherapy and adoptive cell therapy etc. Mostly used treatment techniques are chemotherapy and radiotherapy. Therapeutic utility can be possibly given to nucleic acids by auger electrons by creating breaks in them. The growth of neuroblastoma tumors can be inhibited with the help of sorafenib which targets both neuroblastoma cells and tumor blood vessels. In the nascent stages adoptive immunotherapy can be used for the treatment of neuroblastoma. Many therapies have been researched on and many more are being researched for the treatment of neuroblastoma.

PURPOSE:

To use gene therapy for the treatment of neuroblastoma – a form of pediatric malignancy.

TOOLS FOR DATA COLLECTION:

i. Research papers
ii. Research articles
iii. Discussions
iv. Thesis of various Professors
v. Notes from various meetings held
vi. Questionnaires
vii. Interviews
viii. Reports

DATA ANALYSIS:

Neuroblastoma is a type of pediatric malignancy, characterised by high clinical heterogeneity. It starts from primitive nerve cells found in areas where nerves join together. The tumor regresses spontaneously in infants and on maturation differentiates into benign ganglioneuroblastoma in later stages. The tumor is peripheral and generally present on the sympathethic nervous system. Treating tumors with viruses, that is, oncolytic viruses, was originally suggested by the clinicians who witnessed tumor regression after spontaneous viral infections. An increased understanding of virology, as well as experience using viruses in cancer gene therapy, has prompted a new wave of oncolytic virotherapy. The use of virotherapy against neuroblastoma (NB) is an emerging field. The virus, called adeno-associated virus (AAV) carried the gene for hINF-beta to the liver, which used

that gene to make hIFN-beta continuously at a low level, blocking the growth of blood vessels feeding the tumors.

Gene therapy is the use of DNA as a drug to treat disease by delivering therapeutic DNA into a patient's cells. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene. Other forms involve directly correcting a mutation, or using DNA that encodes a therapeutic protein drug (rather than a natural human gene) to provide treatment. In gene therapy, DNA that encodes a therapeutic protein is packaged within a "vector", which is used to get the DNA inside cells within the body. Cancer occurs by the production of multiple mutations in a single cell that causes it to proliferate out of control. Cancer cells often different from their normal neighbours by a host of specific phenotypic changes, such as rapid division rate, invasion of new cellular territories, high metabolic rate, and altered shape.

Some of those mutations may be transmitted from the parents through the germ line. Others arise de novo in the somatic cell lineage of a particular cell.The cancer patients who are not helped by these therapies may be treated by gene therapy. Neuroblastoma is a type of pediatric cancer . A major goal of tumor immunotherapy is the effective eradication of established metastases associated with the induction of a T cell-mediated protective immunity.

This was achieved in a poorly immunogenic murine neuroblastoma model by gene therapy with a single chain interleukin 12 (scIL-12) fusion protein that assures equal expression of its p35 and p40 subunits. Neuroblastoma originates from neural crest derived cells and most commonly arises from the adrenal medulla or the abdominal sympathetic ganglia. The cancer stem cell hypothesis suggests that many if not all tumours contain a small number of cancer stem cells (CSCs) which express early developmental markers and may act as a reservoir of cancer cells.(Dick JE,et al,2008) Incubation with IL-2(INTERLEUKIN-2) enhances Natural killer (NK) cell cytotoxicity in children with neuroblastoma and in children with acute leukemia.(Casper JT,et al,1992) Limitation to the present clinical use of IL-2 to advanced renal cell carcinoma is since the clinical experience of IL-2 has shown severe toxic effects in children .

IL-l2(INTERLEUKIN-12) is a newly described cytokine that has several properties, including the ability to act synergistically with IL-2 in generating lymphokine-activated killer cells (LAK) against known tumor targets.

MATERIALS AND METHODS

On investigation the role of IL-l2 in the generation of peripheral blood mononuclear cell (PBMC) lysis of neuroblastoma cell lines was established. It was observed that PBMC were activated with IL-12 alone and in combination with IL-2. On continuous experiments it was observed that whereas IL-l2 alone produced only modest enhancement of NK cell cytotoxicity , the most effective method in activating NK cell lysis of neuroblastoma cell lines was the combination of IL-2 and IL-l2 .

Tumor cell lines . Tumor cell lines were maintained by standard cell culture techniques and placed in RPMI-1640 medium (GIBCOLaboratories, Grand Island, NY) containing **10%** heat-inactivated fetal calf **serum** (Hyclone, Logan, UT), 2 mmol/L L-glutamine, **10** U/mL penicillin, 100 IrglmL streptomycin, 5 mmol/L HEPES buffer (GIBCO), and 5 X IO-' mol/L 2-mercaptoethanol (Sigma, St Louis, **MO).** K562 cells, an erythroleukemia cell line, were maintained in suspension. NB100, NB212, and UCH-I cell lines are all adherent neuroblastoma cell lines.The neuroblastoma cell lines were harvested by brief trypsinization with 0.25% trypsin and 0.04% **EDTA** in phosphate-bu&red saline (PBS).

Patient eligibility. Blood samples from three children with neuroblastoma were evaluated. No patient had received chemotherapy in the 2 weeks before evaluation. Informed consent was obtained from each family before removal of blood sample. Few patients were receiving multiagent chemotherapy courses.1/4th of patients was approximately **19** months post autologous BMT. All those patients had stage **IV** neuroblastoma.

PBMC isolation. Fresh leukocyte buffy coats were obtained from healthy donors **or** 1O mL of peripheral blood from patients was obtained. Samples were diluted 1.2 in PBS . The cells were layered over 14 mL of Ficoll-Paque. After centrifugation at 400g for 20 minutes at room temperature, the interface band of PBMC was collected and washed twice with PBS. The collected PBMC were then suspended in RPMI-1640 medium (GIBCO) containing 5% heat-inactivated human AB ***se***rum

NK* cells and eN*a nd T-cell isolation. Fresh buffy coats were obtained as described above and PBMC were further separated using plastic adherence for removal of monocytes followed by further removal of adherent cells and B lymphocytes by incubation on nylon wool columns for 30 minutes at 37°C. The cells passing through the columns were then placed on a six-step discontinuous density gradient with a range from **40%**t o 52.5% Per~oll.'~A fter centrifugation at 550g for 30 minutes at room temperature the bandosf lymphocytes were collected. LGL, obtained from Percoll fractions 2 and 3, were treated with anti-CD5 antibodies and T cells, from fractions 4 through 7, were incubated with anti-CD16 antibodies for 40 minutes. The cells were then incubated with Immunobeads coated with goat antimouse IgG .The labeled cells were removed by exposure to a strong magnetic field. Purified LGL and T cells were then counted and diluted to the appropriate concentration in media with 5% AB serum.

Cytotoxic assay. Cytotoxic activity was evaluated using chromium (5'Cr)r elease assays. Ninety-six-well, U-bottomed microwell plates were prepared with 100 pL, triplicate serial dilutions of PBMC in effector celktarget cell concentrations of 100:1, 50:1,25: I , and 12.5:1. Recombinant IL-2 (Hoffman-LaRoche, Nutley, **NJ)** and IL- 12 (specific activity 8.5 X **1O^7** U/mg) were added to PBMC in concentrations ranging from **1** U/mL to 100 U/mL. The cells were then incubated at 37°C in 5% CO2 for **18** hours. Target tumor cells were counted andw ashed before 100 pCi of sodium" Crchromium (Amersham, Arlington Heights, IL) was added to 2 X **IO^6** cells and incubated at 37°C for I hour.

The cells were washed twice with PBS and diluted to a concentration of 5 X 104/mL, then 100 pL of target cells was added to each well of the 96-well plates. After the cells were incubated at 37'C for 5 hours, 100 pL of supernatant was collected, and the radioactive content was measured by gamma scintillation counting. The percentage of 5'Cr release was calculated.

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