Targeting BRG1 to Improve Drug Efficacy in Neuroblastoma

A Major Qualifying Project

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by _____

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ABSTRACT

Multiple Drug Resistance is one of the main challenges researchers face when developing effective chemotherapy drugs in most cancers. Recent studies have shown that in neuroblastoma, BRG1 gene is active as a pro-oncogenic factor and reducing BRG1 levels by short hairpin RNA decreased neuroblastoma cell proliferation. In a previous study, BRG1 was shown to regulate drug transporter gene expression in response to chemotherapy drug treatment in triple negative breast cancer cells. In this study, we explored the relationship between BRG1 and drug transporters to elucidate the effect of BRG1 silencing on drug resistant neuroblastoma cells treated with chemotherapy drugs. The results support the hypothesis that targeting BRG1 may increase chemo sensitivity in drug resistant neuroblastoma cells.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Mary Lee for allowing me to work in her lab at UMass Medical School, and Dr. Joae Wu for offering her guidance in establishing my project purpose, and for her input in my final report. I would also like to thank Lei Huang for teaching me the many techniques that were involved in the MQP. Finally, a special thanks to Professor Arne Gericke for guiding me through the MQP process, advising me along the way, and for helping in the editing of this final report.

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INTRODUCTION

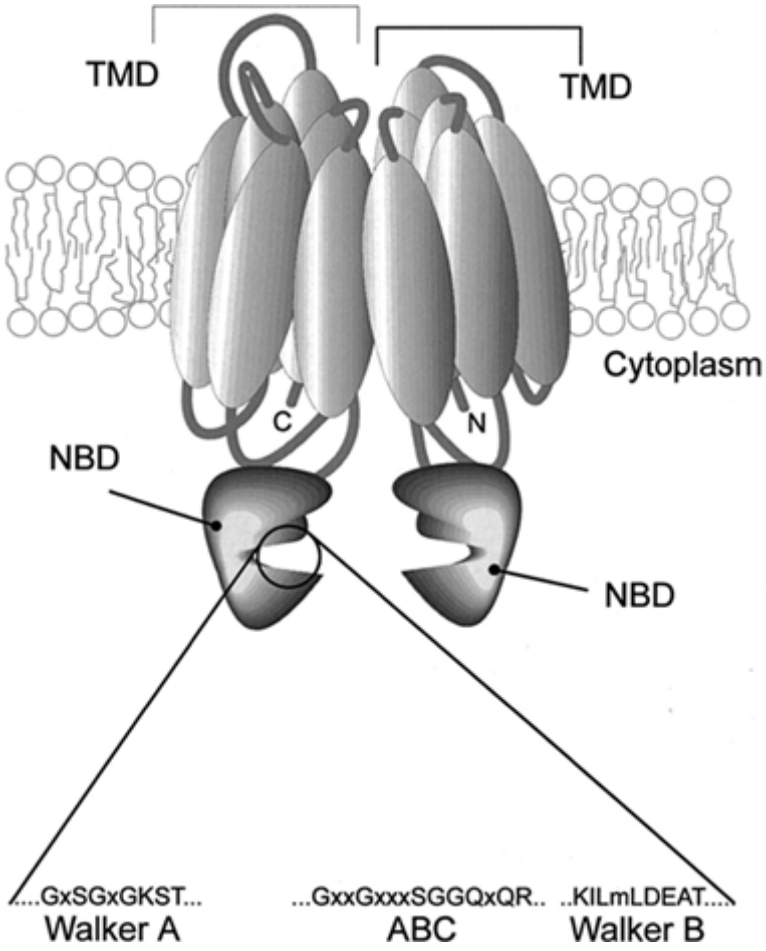
Neuroblastoma (NB) refers to a cancerous disease state in the immature nerve tissue (neuroblasts). It often originates in the neural crest in the adrenal gland, but rarely occur in other sites of the sympathetic chain ganglia from the neck to the coccyx[1]. Typically, this disease is diagnosed in infancy and can even be detected as early as the fetal stage by ultrasounds, though often metastasized by the time it is detected[2]. It is the most common solid tumor found in infants, resulting in about 15% of death every year[3]. NB varies in the potential clinical outcomes, ranging from spontaneous remission to treatment resistant metastatic tumors[3]. Of the high-risk patients who seek treatment in the form of intense rounds of chemotherapy, radiation therapy and bone marrow transplant, about 60-70% of these neuroblastoma cases are not cured. This is because metastatic tumors and relapsed tumors gain resistance to multiple drugs, making it almost impossible to cure using currently known treatments. There are multiple mechanisms that confer such strong therapy resistance to NB, many of which are not yet known.

Drug Transporters Responsible for Multidrug Resistance

Typically, when cancer cells no longer respond to a specific drug, a combination of various drugs that act via different mechanisms will be used. However, cancer cells have the ability to develop resistance to multiple drugs with different mechanisms, a phenomena called multidrug resistance (MDR). This presents a challenge when attempting treatment of cancer by solely depending on drug efficacy[4]. Studies aimed to understand the molecular basis of MDR found that active extrusion of drugs from cancer cells is one of the most common mechanisms employed in developing drug resistance[5]. Many of these systems that carry out drug extrusion harness energy from ATP hydrolysis to transport molecules across cell membranes. The ATP-binding Cassette (ABC) family ATPases are the most well characterized drug transporters. These ABC transporters

have two transmembrane domains and two nucleotide binding domains which are found on the cytoplasmic membrane surface where the ABC signature sequence is located as seen in Figure 1 below[5].

Figure 1



From: Mechanisms of multidrug transporters FEMS Microbiol Rev. 1997;21(1):55-84. doi:10.1111/j.1574-6976.1997.tb00345

In human cancer cells, P-glycoprotein (P-gp or MDR1) encoded by ABCB1 gene, multidrug resistance proteins (MRPs) encoded by ABC C family members and ABCG2 are some of the main ABC transporters are known to confer multidrug resistance[6,7]. The MRP genes include MRP1 which

is an integral membrane protein which a core that is similar to Mdr-1, it is commonly expressed in various cells including cerebral cells. MRP4 and MRP5 are also expressed in various cells types at lower levels and are encoded by ABCC4 and ABCC5 respectively. MDR1is a protein that functions as a drug transport pump located in the blood-brain barrier shuttling various drugs from the brain back into the blood. The MDR1 gene is located on chromosome 7 and also referred to as ABCB1[8]. ABCG2 is abundantly expressed in placenta, intestine and stem cells. It is a half-transporter that provides resistance to a variety of chemotherapeutic drugs including mitoxantrone, bisantrene and anthracyclines such as doxorubicin[9]. ABCG2 is essential for stem cell differentiation and under normal conditions, and is also found in the blood-brain barrier where it is responsible for disposing of toxins found in cells[7]. However, it is still unclear to researchers which particular cancers need these transporters in an effort to identify them as clinical targets for possible therapies[7]. In this study, I surveyed the expression of these transporter genes in drug resistant CHLA-20 and SK-N-BE(2) cell lines and measured the chemotherapy drug efficacy in these cells with or without BRG1 knockdown.

BRG1 AND NB

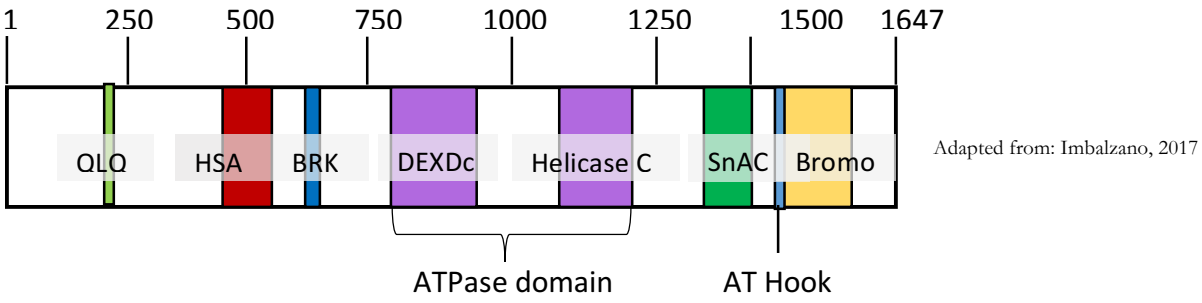


Figure 2. Domain Structure of BRG1

The domains that are conserved are labelled and the numbers across the top represent amino acids

When DNA is tightly packed, it is not accessible for transcription. The mammalian SWI/SNF complex is a chromatin remodeling complex that uses the energy of ATP hydrolysis to open up the tightly packed DNA sequences for transcription. The mammalian SWI/SNF complex contains two mutual exclusive ATPases, BRG1 encoded by SMARCA4 or BRM encoded by SMARCA2. Many cellular processes including cell proliferation, transcription, immunological responses, early development of an embryo, cancer and tissue differentiation are regulated by the SWI/SNF complex. As seen above, BRG1 is a multi-domain protein that shares about 86% similarity with BRM, another ATPase subunit. BRG1 has both DNA and protein interaction modules as seen in Figure 2. In eukaryotic cells, DNA is not present as a linear double helix. Instead, DNA is tightly coiled around histones to form nucleosomes, and nucleosomes pack together to form chromatin.

While BRG1 was previously identified as a tumor suppressor in some cancers[10], overexpression has also been associated with poor outcomes for patients and upregulated in multiple carcinomas including melanoma[11], pancreatic cancer[12], glioma[13], prostate carcinoma[14,15] and breast cancer[16]. A recent study suggests that BRG1 may, in fact, be necessary for proliferation of NB cells and BRG1 gene silencing can interrupt cell proliferation and tumor growth. BRG1's role proved to be essential in neuroblastoma; knockdown led to reduction in cell growth, apoptotic cell death and slowed tumor progression in mouse xenograft model[3]. In breast cancer, it was shown that BRG1 was responsible for the upregulation of *de-novo* fatty acid synthesis in an effort to meet the demand of cancer cell proliferation[17]. Further, BRG1 is required to up-regulate drug transporter gene expression to facilitate cancer cells survival under chemotherapy treatment. Based on its essential roles in neuroblastoma and breast cancer, we hypothesize that BRG1 may involve in mediating chemotherapy resistance in neuroblastoma.

RESULTS

1. Survey drug transporter gene expression

   We used four pairs of neuroblastoma cell lines that derived from patients' pretreated tumors and post-treatment but relapsed tumors. The CHLA-15, SK-N-BE(1), SMS-KCN and SMS-KAN pretreated cell lines were paired with CHLA-20, SK-N-BE(2), SMS-KCNR and SMS-KANR relapsed tumor cell lines respectively (Appendix). The matched pair of cell lines were derived from the same patient. We surveyed the transporter gene expression by RT-qPCR in these lines. Relative gene expression was measured by comparing the expression of transporter genes to the house keeping gene GAPDH and the fold change was presented as the relative gene expression in relapsed lines to pre-treatment lines. In figure 3, it is important to note that there is an overall increase in the expression of these drug transporters in the relapsed tumor cell lines.

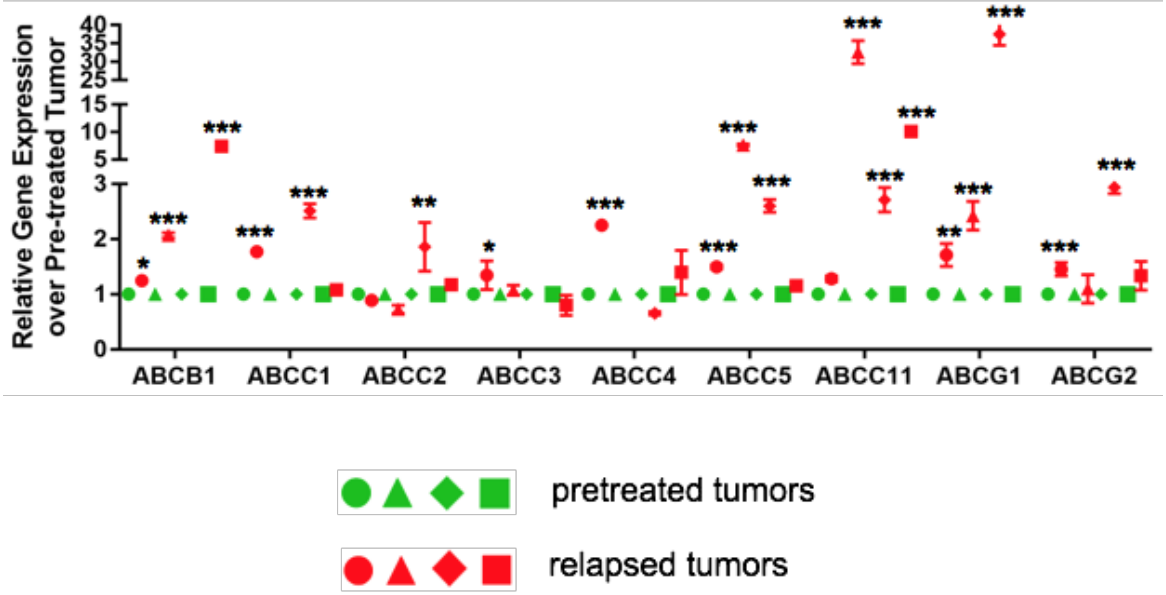


Figure 3. Drug Transporters Are Up-regulated in Relapsed NB

2. Chemotherapy drug treatment induced the expression of transporter genes

In pre-treated neuroblastoma line CHLA-15 responded to etoposide treatment with an IC90 at 0.2 ng/mL. However, in CHLA-20 relapsed tumor cells there is a development of resistance to etoposide (ETOP) and effective dose of etoposide increased to 691 ng/mL. Etoposide treatment at 350 ng/mL in CHLA-20 dose markedly increased the expression of major drug transporters such as ABCB1, ABCC1, ABCC3, ABCC4, ABCC5, ABCC11 and ABCG1 (Figure 3). It is worth noting that in addition to an increase in some drug transporter expression, BRG1 expression was also increased in the presence of this chemotherapy drug. In cells that normally express P-gp, there is typically an increase in P-gp expression following chemotherapy and can result in multidrug resistance in some tumors[24]. Multidrug resistance is often acquired as a result of various transporters preventing several drugs from being effective, this is supported by the increase in other transporter mRNA levels following ETOP treatment.

3. Generation of doxycycline inducible BRG1 knockdown lines in CHLA-20 and SK-N-BE(2)

To better understand the role of BRG1 in chemotherapy resistant neuroblastoma cells, we engineered short hairpin RNA (shRNA) either scramble control or shRNA targeting BRG1 in CHLA-20 and SK-N-BE(2) cells in doxycycline inducible manner. In the absence of doxycycline, the shRNA which is under the control of tetracycline repressor will not be expressed thus there is no BRG1 knockdown effect. Only in the presence of doxycycline, the shRNA will be de-repressed and subsequently interfere with BRG1 expression to achieve a gene knockdown effect. As seen in Figure 4 below, Western blotting confirmed the successful knockdown of BRG1 expression in these two cell lines.

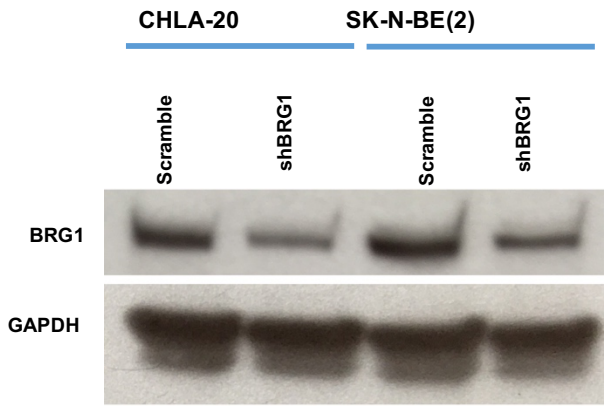


Figure 4. Western Blot to confirm successful BRG1 gene knockdown in shBRG1 cells

4. BRG1 knockdown sensitized resistant neuroblastoma cells to chemotherapy

To test the hypothesis that reducing BRG1 levels enhances chemotherapy efficacy, we treated the pre-treatment line SK-N-BE(1), post-treatment and relapsed lines SK-N-BE(2) scramble or SK-N-BE(2)shBRG1 with chemotherapy drug doxorubicin. SK-N-BE(1) cells are sensitive to doxorubicin with IC90 <0.1 ng/mL, whereas SK-N-BE(2) cells are resistant to doxorubicin with IC90 at 92.3 ng/mL. As shown in Figure 6, knockdown of BRG1 significantly enhanced doxorubicin efficacy in the resistant cells almost to the level of pre-treatment cells.

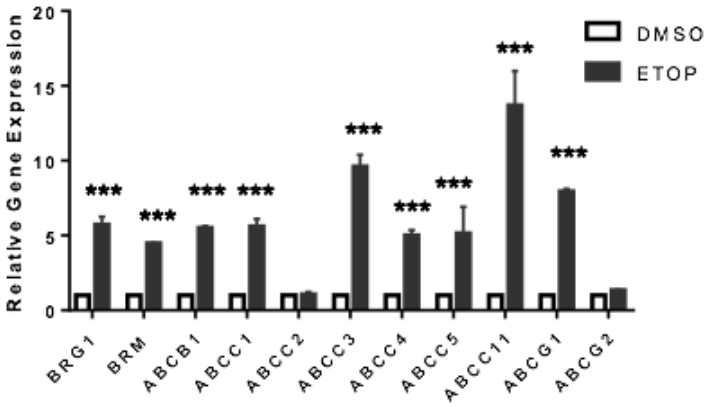


Figure 5. Etoposide drug-induced ABC transporter gene expression in neuroblastoma cell line CHLA-20

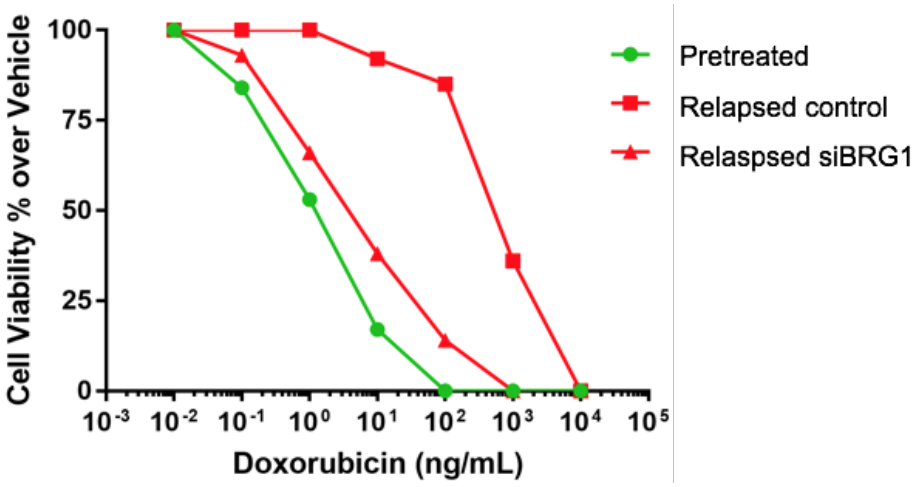


Figure 6. Reduction of BRG1 levels in relapsed cell lines increased the chemo sensitivity of neuroblastoma cells to doxorubicin

Research shows that BRG1 regulates some drug transporters in cancer cells which are responsible for chemotherapy drug trafficking[4]. BRG1 was discovered to be bound near the transcription start sites for multiple ABC transporters, suggesting that BRG1 plays a role in drug-mediated up-regulation of these transporters[4]. To determine the effect that BRG1 knockdown has on sensitivity to drug treatment, the BRG1 gene was silenced using siRNA. Figure 6 shows that following BRG1 gene silencing in the relapsed tumor cell line, these formerly drug resistant cells now have a sensitive response to doxorubicin treatment.

DISCUSSION

Multiple drug resistance has always been a challenge in cancer treatment via chemotherapy and many years of research have been done to develop an effective approach to overcome drug resistance. Over-expression of drug transporters such as MDR1 or P-gp is one of the most common cause of drug resistance. Therefore, these transporters became attractive target and many inhibitory compounds were developed over the last decades. However, these transporters also play important roles in normal physiology. This led to the failure of clinical trials of inhibitors directly targeting these transporters.

We have shown that the expression of drug transporter genes is much higher in drug resistant neuroblastoma cells compared to pre-treated cells. Chemotherapy drug treatment significantly increased the expression of these transporters in drug resistant cells. More importantly, BRG1 knockdown enhances chemotherapy drug efficacy in drug resistant cells. Therefore, targeting BRG1could be an effective approach to overcome drug resistance in neuroblastoma. ABC transporters which are usually responsible for drug extrusion in various cancer cells depend on BRG1[26]. This suggests that silencing BRG1 should result in a reduction of drug extrusion leading to decreased chemotherapeutic failure. Research has proved that BRG1 is essential in NB cells viability and silencing BRG1 promoted NB cells to apoptosis. Since BRG1 has been shown to involve in other signaling pathways such as lipogenesis, WNT signaling, and DNA repaired, it is possible that other mechanisms are contributed to the increased drug efficacy when BRG1 is silenced[26]. This provides a promising path into treating cancers like neuroblastomas specifically by inhibiting BRG1 in order to return drug sensitivity to relapsed tumor cells enhancing clinical outcomes of chemotherapy. I conclude that these findings present the possibility that targeting BRG1 could be an effective way to increase the effectivity of chemotherapy drug use in treating neuroblastoma

patients. Though challenging, this is a promising place for further research on whether specific BRG1 knockdown is a reliable solution that can actually be used to treat tumors in patients.

MATERIALS AND METHODS

Cell Culture

The human neuroblastoma cell lines (CHLA-15, CHLA-20, SK-N-BE(1), SK-N-BE(2), SMS-KCN, SMS-KCNR, SMS-KAN, and SMS-KANR) were obtained from Childhood Cancer Repository from the Children's Oncology Group Resource Library. CHLA-15 and CHLA-20 cells were grown in a base medium of Iscove's Modified Dulbecco's Medium plus the following supplements (to a final concentration): 20% Fetal Bovine Serum, 4mM L-Glutamine, 1X ITS (5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL selenous acid). SK-N-BE(1), SK-N-BE(2), SMS-KCN, SMS-KCNR, SMS-KAN, and SMS-KANR cells were cultured in RPMI-1640 media plus 10% Fetal Bovine Serum and 2mM L-Glutamine . Al cells Cells were cultured in 10cm dishes and incubated at 37°C in a humidified atmosphere of 5% CO2.

Lentiviral mediated gene knockdown

The doxycycline inducible SMART Vector scramble and shBRG1 were purchased from Dhamarcon Inc. The lentivirus cDNAs were produced by co-transfecting with plasmids pLP1, pLP2 and pVSVG (Invitrogen) into HEK293T cells. The viral supernatant was collected 48 hours after transfection and transduced the cells for 24 hours followed by selection in media containing 2 ug/mL puromycin. Titers were determined and the same number of virus was used, as indicated. Gene knockdown was induced by incubation of cells in medium containing 1μg/mL Doxycycline (Sigma-Aldrich) for 72 hours.

Drug Treatment

Cells were plated at 5,000 cells/per well in 96-well plates and incubated overnight before treatment with increasing doses of drugs for 72 hours.

MTT assay

Cell viability was measured using MTT assay. Cell viability was measured in 24 hour intervals by adding MTT solution (final concentration 5 μg/mL) to each well and incubated for 4 hours. After removal of the media, the plate was air-dried and 100 μL DMSO were added. The plate was incubated at room temperature for 30 min with gentle shaking. Absorbance was measured at OD540 in a Synergy H4 Hybrid microplate reader (Bio Tek, Winooski, VT).

RT-qPCR

Total RNA was extracted from one million cells using RNeasy Plus following manufacturer's instruction (Qiagen Inc., Valencia, CA). cDNA synthesis was done using SuperScript III kit (Invitrogen, San Diego, CA). Gene expression was measured by real time qPCR on a StepOne Plus realtime PCR System (Applied BioSystems, Grand Island, NY) using various primers.

Statistical Analyses

Quantitative data points represent the mean of three independent experiments performed in duplicates or triplicates with standard deviation (S.D). Unless indicated, statistical analysis was performed using GraphPad Instat two-tail P value student test (Graphpad Software, Inc., La Jolla, CA). The significance of the correlation between BRG1 expression and the expression of each of the transporter genes was determined by calculating the Pearson coefficient.

APPENDIX

CHILDREN'S ONCOLOGY GROUP

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER
School of Medicine
Cancer Center

CHLA-15 Cell Line Data Sheet

Cell Line Name: **CHLA-15**

Disease: Neuroblastoma
Phase of Therapy: Diagnosis
Prior Therapy: None
Disease Stage: 4
Source of Culture: Primary tumor
Primary Tumor Site:
Date Established: April 1988

MYCN Status: Non-amplified
p53 Status: Functional
TH expression: Expressed
Karyotype:
Modal No:

IC90 (DIMSCAN*):
***see reference 7**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
1.1	0.2	1.1	0.2	1.5

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Female
Age: 18 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of Iscove's Modified Dulbecco's Medium plus the following supplements (to a final concentration): 20% Fetal Bovine Serum, 4mM L-Glutamine, 1X ITS (5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenous acid)

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 21 hours
Morphology: Teardrop-shaped cells with processes
Growth Properties: Adherent, grows as loosely attached monolayer, numerous tight clumps

Notes: COGcell.org has a post-treatment cell line available from this same patient (CHLA-20)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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CHLA-20 Cell Line Data Sheet

Cell Line Name: **CHLA-20**

Disease: Neuroblastoma
Phase of Therapy: Post-Chemotherapy (Progressive Disease)
Prior Therapy: Cisplatin, cyclophosphamide, doxorubicin, teniposide
Disease Stage: 4
Source of Culture: Primary tumor
Primary Tumor Site:
Date Established: October 1988

MYCN Status: Non-amplified
p53 Status: Functional
TH expression: Expressed
Karyotype:
Modal No:

IC90 (DIMSCAN*):
***see reference 5**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
3.7	1.1	497.6	691	1.3

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Female
Age: 24 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of Iscove's Modified Dulbecco's Medium plus the following supplements (to a final concentration): 20% Fetal Bovine Serum, 4mM L-Glutamine, 1X ITS (5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenous acid)

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 28 hours
Morphology: Teardrop-shaped cells with processes
Growth Properties: Adherent, grows as loosely attached monolayer, numerous tight clumps

Notes: COGcell.org has a diagnosis (pre-therapy) cell line available from this same patient (CHLA-15)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SK-N-BE(1) Cell Line Data Sheet

Cell Line Name: **SK-N-BE(1)**

Disease: Neuroblastoma
Phase of Therapy: Diagnosis
Prior Therapy: None
Disease Stage: 4
Source of Culture: Bone marrow
Primary Tumor Site:
Date Established: June 1972

MYCN Status: Amplified
p53 Status:
TH expression: Expressed
Karyotype:
Modal No: 46

IC90 (DIMSCAN*):
***see reference 7**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
0.2	<0.1	<0.1	158	0.8

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Male
Age: 24 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 96 hours
Morphology: Neuroblasts with some attached containing occasional processes
Growth Properties: Adherent and suspended cells

Notes: COGcell.org has a post-treatment cell line available from this same patient - SK-N-BE(2)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SK-N-BE(2) Cell Line Data Sheet

Cell Line Name: **SK-N-BE(2)**

Disease: Neuroblastoma
Phase of Therapy: Post-Chemotherapy (Progressive Disease)
Prior Therapy: Cyclophosphamide, doxorubicin, vincristine, local irradiation
Disease Stage: 4
Source of Culture: Bone marrow
Primary Tumor Site: Brain
Date Established: November 1972

MYCN Status: Amplified
p53 Status: Non-functional
TH expression: Expressed
Karyotype: t(1;2)(p22;p21),t(3;17)(p21;q21), Monosomy (17,18), variable rearrangements involving chromosomes (4,6,9,10,11,and/or 20)
Modal No: 44

IC90 (DIMSCAN*):
***see reference 9**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
2.1	0.2	92.3	1130	24

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Male
Age: 24 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 30 hours
Morphology: Neuroblasts
Growth Properties: Adherent and suspended cells

Notes: COGcell.org has a diagnosis (pre-therapy) cell line available from this same patient - SK-N-BE(1)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SMS-KCN Cell Line Data Sheet

Cell Line Name: **SMS-KCN**

Disease:	Neuroblastoma
Phase of Therapy:	Diagnosis
Prior Therapy:	None
Disease Stage:	4
Source of Culture:	Primary tumor – retroperitoneum, liver (anterior apron), lymph nodes (portal hepatis)
Primary Tumor Site:	Adrenal gland
Date Established:	September 1979
MYCN Status:	Amplified
p53 Status:	Functional
TH expression:	Expressed
Karyotype:	del(1)(p34), t(17;20)(q21or22;q13)
Modal No:	46

IC90 (DIMSCAN*):
***see reference 6**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
1.4	0.5	24.7	323	5.5

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender:	Male
Age:	11 months
Race:	Caucasian
STR Profile:	On file at http://strdb.cogcell.com
Media Formulation:	Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine
Growth Conditions:	37.0°C, 5% CO_2, 20% O_2
Subculturing:	Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time:	109 hours
Morphology:	Round, teardrop-shaped neuroblasts
Growth Properties:	Adherent and suspended cells, grow mostly in clumps
Notes:	COGcell.org has a post-treatment cell line available from this same patient (SMS-KCNR)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SMS-KCNR Cell Line Data Sheet

Cell Line Name: **SMS-KCNR**

Disease:	Neuroblastoma
Phase of Therapy:	Post-Chemotherapy (Progressive Disease)
Prior Therapy:	Doxorubicin, cyclophosphamide
Disease Stage:	4
Source of Culture:	Bone marrow
Primary Tumor Site:	Adrenal gland
Date Established:	November 1979
MYCN Status:	Amplified
p53 Status:	Functional
TH expression:	Expressed
Karyotype:	del(1)(p34), t(17;20)(q21or22;q13), t(6;?)(q21or22;?)
Modal No:	46

IC90 (DIMSCAN*):
***see reference 11**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
1.9	0.3	17	10.5	3.8

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender:	Male
Age:	11 months
Race:	Caucasian
STR Profile:	On file at http://strdb.cogcell.com
Media Formulation:	Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine
Growth Conditions:	37.0°C, 5% CO_2, 20% O_2
Subculturing:	Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time:	72 hours
Morphology:	Round, teardrop-shaped neuroblasts
Growth Properties:	Adherent and suspended cells, grow mostly in clumps
Notes:	COGcell.org has a diagnosis (pre-therapy) cell line available from this same patient (SMS-KCN)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SMS-KAN Cell Line Data Sheet

Cell Line Name: **SMS-KAN**

Disease: Neuroblastoma
Phase of Therapy: Diagnosis
Prior Therapy: None
Disease Stage: 4
Source of Culture: Primary tumor
Primary Tumor Site: Pelvis
Date Established: October 1978

MYCN Status: Amplified
p53 Status:
TH expression: Expressed
Karyotype: del(1)(p21),del(4)(q21),del(11)(q21),del(17)(p11),del(19)(q13),t(4;10)(q21;q26),t(17;18)(q21;p11)44
Modal No: 46

IC90 (DIMSCAN*):
***see reference 9**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
2.2	0.3	34.8	146	0.6

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Female
Age: 36 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 95 hours
Morphology: Round, teardrop-shaded neuroblasts
Growth Properties: Adherent and suspended cells, grow mostly in clumps

Notes: COGcell.org has a post-treatment cell line available from this same patient (SMS-KANR)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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SMS-KANR Cell Line Data Sheet

Cell Line Name: **SMS-KANR**

Disease: Neuroblastoma
Phase of Therapy: Post-Chemotherapy (Progressive Disease)
Prior Therapy: Cyclophosphamide, doxorubicin, radiation therapy
Disease Stage: 4
Source of Culture: Bone marrow
Primary Tumor Site: Pelvis
Date Established: October 1978

MYCN Status: Amplified
p53 Status:
TH expression: Expressed
Karyotype: del(1)(p21),del(19)(q13),del(4),del(11),del(17),t(4;10),t(17;18),t(11;?)(q23;?),t(13;?)(p11or13;?), inv(7)(p22q11.2),t(6;?)(q21or22;?),Monosomy (8,14,88,22),3 unidentified markers
Modal No: 46

IC90 (DIMSCAN*):
***see reference 6**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
1.3	0.8	45.7	45.7	5.6

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Female
Age: 36 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 69 hours
Morphology: Round, teardrop-shaded neuroblasts
Growth Properties: Adherent and suspended cells, grow mostly in clumps

Notes: COGcell.org has a diagnosis (pre-therapy) cell line available from this same patient (SMS-KAN)

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

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