Minireview *Highlight article*

An overview of neuroblastoma cell lineage phenotypes and *in vitro* models

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Impact statement

This review provides an update on the mostly used cell line *in vitro* models for neuroblastoma (NB), a heterogeneous disease with high metastatic potential and resistance to treatment. The genetic and phenotypic profiles of the most used NB cell lines in the last 10 years are presented, considering the molecular markers that are involved in the distinct NB tumor phenotypes, including distinct core regulatory circuitries and non-coding RNAs. This gathered information can assist in the selection of the most appropriate NB *in vitro* model, based on the specific goals and objectives of each study.

Abstract

This review was conducted to present the main neuroblastoma (NB) clinical characteristics and the most common genetic alterations present in these pediatric tumors, highlighting their impact in tumor cell aggressiveness behavior, including metastatic development and treatment resistance, and patients' prognosis. The distinct three NB cell lineage phenotypes, S-type, N-type, and I-type, which are characterized by unique cell surface markers and gene expression patterns, are also reviewed. Finally, an overview of the most used NB cell lines currently available for *in vitro* studies and their unique cellular and molecular characteristics, which should be taken into account for the selection of the most appropriate model for NB pre-clinical studies, is presented. These valuable models can be complemented by the generation of NB reprogrammed tumor cells or organoids, derived directly from patients' tumor specimens, in the direction toward personalized medicine.

Keywords: Pediatric cancer, neural crest tumors, neuroblastoma cell lines, SH-SY5Y, tumor stem cells, *in vitro* models

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Introduction

Neuroblastoma (NB) is an extracranial solid tumor in children and comprehends 8% to 10% of all pediatric cancers.[1] It is a heterogeneous disease that presents a broad spectrum of clinical behaviors; in children aged 18 months or older, it is often unresectable or metastatic, requires intensive multimodal therapy, and is associated with a survival rate lower than 50%.[2] On the other side of the spectrum, NB with spontaneous regression without chemotherapy is seen in low-risk subgroups.[3]

NB is derived from cells within the neural crest, likely sympathoadrenal progenitor cells that differentiate to sympathetic ganglion and adrenal catecholamine-secreting chromaffin cells.[4] The presentation and symptoms at diagnosis reflect the tumor location, commonly in the adrenal medulla or anywhere along the sympathetic ganglia. Metastases are present in about 50% of patients at diagnosis, with bone marrow metastases corresponding to 80% of the cases. Metastases are also found in bones and regional lymph nodes, while the involvement of the central nervous system and lungs is rare.[5]

The International Committee of Pathology of Neuroblastoma classifies NB tumors into distinct subtypes, according to histological findings, which include the amount of Schwannian stroma present in the tumor and the mitosis-karyorrhexis index (MKI).[6] Generally, the poorly differentiated or undifferentiated histology confers a worse prognosis to patients. Age is also an important prognostic indicator; in <18-month-old patients, poorly differentiated NB is still considered a favorable prognosis if the MKI is not high; however, in patients aged >18 months, poorly differentiated NB is invariably unfavorable.[7]

DNA or ploidy index, *MYCN* oncogene amplification status, and specific segmental chromosomal aberrations also influence NB prognosis,[8] as it will be discussed later in this review. Furthermore, nerve growth factor (NGF) and other neurotrophins (NT) play important roles in the survival, differentiation, and activity of NB cells, mediated by tyrosine-kinase receptors (TrkA, TrkB, and TrkC).[9] Favorable prognostic tumors usually express TrkA, the NGF receptor, while unfavorable prognostic tumors, with amplification of the *MYCN* oncogene, usually express TrkB and the ligand brain-derived neurotrophic factor (BDNF).[10,11] The TrkB-BDNF signaling pathway seems to function in an autocrine mode of action, increasing survival in NB cells and resistance to chemotherapy, via both the PI3K/Akt and the p44/42 MAPK signaling pathways, according to the chemotherapy drug used.[12]

NB most common genetic alterations

Although NB is the most common embryonic malignancy diagnosed during the first year of life,[13] hereditary NB tumors occur in less than 1% of cases,[14] where a few inherited mutations, such as the ones in *ALK* and *PHOX2B* genes, were reported to confer tumor predisposition.[15,16] In sporadic tumors, somatic mutations are not common[17]; however, chromosome aberrations are frequent and were shown to directly correlate with tumor aggressiveness and progression.[18] These aberrations, which are critical risk factors previously identified in primary NB, can involve amplification of the *MYCN* oncogene, mutations of the *ALK* receptor, allelic deletions on the 1p and 11q chromosome regions, whole chromosome gains, and gains on the 17q chromosome region.[13,19–21]

MYCN

Amplification of the proto-oncogene *MYCN* (MNA) is present in about 20%–25% of NB tumors, especially in high-risk patients.[22] MNA was described in the early 1980s as a tumor aggressiveness factor,[23] and it is still considered the most widely used genetic marker for NB clinical prognosis.[22,24] *MYCN* is located on chromosome 2p24 and encodes a phosphoprotein of the MYC family of transcription factors, which binds to specific promoter sequences, favoring cell proliferation.[25] *MYCN* targets the proto-oncogene *MDM2*, a ubiquitin protein ligase which directly regulates the p53 protein[26]; the TP53 tumor suppressor gene is rarely mutated in NB[27]; however, the inhibition of the p53 pathway may occur through *MYCN* amplification, which can lead to the high expression of MDM2 protein. Overexpression of the *ALK* gene, also located on chromosome 2, in association with MNA, may also be found in sporadic NB driven by mutations, which are often identical to those identified in familial NB.[16,28]

Chromosome 1p. Chromosome 1p deletions occur in 23% to 46% of NB tumors and are associated with poor prognosis and metastatic disease.[24,29,30] The commonly deleted region is on 1p36, which contains many genes associated to NB malignant behavior, including *CHD5*, *TNFRSF25*, *CAMTA1*, *AJAP1*, *ERRFII*, *PIK3CD*, *RBP7*, and *TARDBP*.[31–33] Among these, *CHD5* was shown to be the most critical tumor suppressor gene in this chromosome region.[34] A high *CHD5* expression is associated with a favorable prognosis; however, deletion or low expression of *CHD5* is frequently observed in high-risk tumors.[35] CHD5 is a member of the chromatin remodeling gene family, encoding a protein with chromatin-remodeling, helicase, and DNA-binding motifs (CHD), with preferential expression in neural tissues, directly associated with nervous system development.[36] It controls cell proliferation, apoptosis, and senescence through p19 and p53 pathways regulation[35,37] and can be upregulated by the treatment with retinoic acid (RA), inducing cell differentiation via NGF/TrkA signaling.[38] RA is a well-known differentiating agent reported to reduce stemness characteristics[39] and treatments based on RA are currently used for high-risk NB.[14,40]

Chromosome 11q. Deletions on chromosome 11q (del11q-q25) are present in 17% to 48% of NB tumors, depending of the cytogenetic method used for its detection.[24,29,41–43] The main consequence of this aberration is the increase of genetic instability, leading to gene mutations and deregulation of the cell cycle.[44] Its presence is also related to a less favorable outcome and with an increased probability of disease recurrence, even in patients with localized tumors.[45] This poor outcome reinforces the relevance of the inclusion of the 11q chromosome status as one of the criteria for NB risk classification.

Although several molecular studies have concentrated on the identification of cancer driver genes on the 11q region, which includes *CADM1*, *ATM*, *H2AFX*, *PHOXA2*, *SDHS*, *CCND1*, *NCAM*, and *CHK1* genes, the specific targets of this region that present key relevance to NB are still largely unknown.[43,46,47] The *CADM1* gene (also known as *TSLC1*, *NECL-2*, *IGSF4*, and Syn*CAM1*), located at 11q23.3, for example, is a suppressor gene altered in multiple types of cancer.[48] The CADM proteins family is reported to be involved in cell-cell adherence, potentially playing a role in epithelial–mesenchymal transition (EMT)-like processes and tumor progression.[49] In NB, the hypermethylation of *CADM1* was correlated with reduced levels of mRNA and protein expression, in association with lower event-free and overall survival.[50] On the other hand, the overexpression of CADM1 in NB cells was shown to impair cell proliferation and viability as well as colony formation.[51]

Chromosome 17q. Gains on chromosome 17q are the most frequent chromosome aberrations in NB tumors,[52] with a frequency that varies from 23% to 70%, often associated with age at diagnosis and advanced stages of the disease.[20] Although the accurate frequencies and boundaries of the chromosomal copy number variations on 17q and the molecular targets affected, have yet to be identified,[21] gains extending from 17q23.1 to 17qter region have been described as promoting tumor progression.[52] In addition, gains on 17q are positively correlated to MNA,[53,54] and combined with mutations in the *PHOX2B* gene, are

considered to be critical for the acquisition of NB metastatic potential.[55]

NB cell lineages phenotypes

In 1947, Murray and Stout described the common characteristics of neuroblasts derived from eight pairs of primary and metastatic tumors and their morphological changes in two-dimensional culture.[56] The cells, also referred to as sympathicoblasts, produced neurites of varying lengths, some of which begun to branch, acquiring a filamentous shape after four days in culture. It is now known that NB-derived cells are, in fact, heterogeneous and, when cultivated *in vitro*, represent populations with distinct morphological and tumorigenic properties, which reflect the biological and genetic specificities of original neuroblasts.[57,58] At least three different NB cellular phenotypes have been identified: neuroblastic (N-type), flat or substrate adherent (S-type), and intermediate (I-type), each of them presenting particularities that can be identified by specific molecular markers (Table 1).

The origin of these different phenotypes is still controversial, but there are two hypotheses. The first and most accepted hypothesis is known as transdifferentiation theory, which suggests that NB cells are capable of spontaneously interconverting from one phenotype to another.[59] According to this hypothesis, S-type and N-type cells originate from the undifferentiated I-type cells,[59–61] which could explain the appearance of S-type cells during the culture of N-type cell lines, or vice-versa, when I-type cells are still present. The clonal expansion theory, on the other hand, considers that both N and S-type cells could coexist in culture, and that, under certain conditions, the less-aggressive S-type could dominate the cell population over the more aggressive N-type cells.[62,63]

Regardless of the theory, it is clear that an NB cell lineage can contain a mixture of cell types, even if one is more predominant than the others. It is also important to consider that culture conditions can lead to new interactions between these cell types and contribute to distinct responses to a given tested stimuli or treatment. The activation of NOTCH pathway[61] and hypoxia factors,[64] for example, have recently been described to be involved in the differentiation processes of these NB cells, although others may exist as well.

Table 1. Main phenotype markers for neuroblastoma cell lineages.

CELL MARKERS	N	S	I
S-type			
Vimentin	−	+	−/+
CD44	−/+	+	−/+
Fibronectin	−/+	+	−/+
Dopamine beta-hydroxylase	+	−	−/+
N-type			
Neurofilaments	+	−	−/+
β-tubulin	+	−	−/+
I-type			
CD-133	−	−	+
c-KIT	−	−	+

Note: (+) High expression; (−/+) Moderate to low expression; (−) Rare expression.

N-type cells

N-type cells are characterized as immature nerve cells, precursors to the neural crest sympathoadrenal lineage. In culture, they are small and rounded, with thin and long neurofilaments and a scant cytoplasm.[65–67] Although they present a high proliferation rate, form cell aggregates, and adhere weakly to the substrate,[68–70] their tumorigenicity is moderate.

β-tubulin heterodimer, a component of microtubules in neuronal cells, has been used as a specific marker for the highly proliferative N-type cells (Table 1).[67] The dynamic microtubule structures of this component are involved in cell movement and maintenance of cell shape, intracellular trafficking, and cell mobility during mitosis and meiosis.[71] In the context of cancer, tubulin proteins are targets for tubulin-binding chemotherapeutics, which leads to mitotic arrest and cell death.[72,73] Changes in microtubule stability and expression of their isotypes have been correlated with a poor prognosis and chemotherapy resistance in several cancer types, including NB.[74,75]

Neurofilaments represent the main structural component of the cytoskeleton in mature neurons, determining axon caliber and conductivity for proper neuronal function.[76] They are highly present in tumors of neuronal origin, and therefore, are largely used to characterize N-type cells,[65,66,77] even considering that they present a higher expression in benign or differentiated tumors than in malignant and less differentiated tumors of neuronal lineage.[77–81]

Dopamine β-hydroxylase (Dβ-H) is another highly expressed marker in N-type cells with low expression in the S- or I-types.[66,82–84] Dβ-H is an enzyme that characterizes the neuronal differentiation of neuroblasts[83] and is involved in the conversion of dopamine to norepinephrine, released with other vesicular contents during synaptic transmission.[85]

S-type cells

S-type cells are multipotent precursors to melanocytes, Schwann, and central nervous system glial cells that represent the neural crest non-neuronal components. They are characterized as non-tumorigenic and present a large and flat morphology with extensive cytoplasm, few and short neurofilaments, and a large cytoplasmic/nuclear ratio.[66,86] In culture, S-type cells are slow-growing, grow in a monolayer, and are specially characterized by their strong ability to adhere to the substrate.[69] The high expression of β-integrin, which provides a strong attachment to extracellular matrix (ECM) components, along with low levels of insulin-like growth factor receptor, may contribute to the less tumorigenic and less migratory phenotype of these cells.[87]

CD44 is another cell surface receptor commonly used as marker of the NB S-type cells.[88] It binds to hyaluronic acid and many other ECM components and acts as a co-receptor

Table 2. Clinical, biological and genetic features, cell lineage phenotypes and related cell lines of the most used NB cell lines.

Cell line	Gender	Age	INSS stage	Origin site	Treatment	Phenotype	Mycn status	Ch 1p status	Ch 11q status	Ch 17q status	Related cell lines	References
SK-N-SH	F	4 y	4	Bone marrow	CT/RT	I	Non-amplified	Normal	Normal	Gain	Sublines SH-SY5Y/SH-EP	68,130,132,133
BE(2)-C	M	25 mo	4	Bone marrow	CT/RT	I	Amplified	–	–		Parental SK-N-BE(2)	65,89,130,134,135
NB9	M	22 mo	4	Adrenal gland	None	I	Amplified	Loss	Normal	–	–	130,132,136
SH-SY-5Y	F	4 y	4	Bone marrow	CT/RT	N	Non-amplified	Normal	Normal	Gain	Parental SK-N-SH	65,67,126,133
SK-N-BE (1)	M	20 mo	4	Bone marrow	None	N	amplified	Loss	-	Normal	SK-N-BE (2)	65,128,130,134,135
SK-N-BE(2)	M	24 mo	4	Bone marrow	CT/RT	N	Amplified	Loss	Normal	Gain	Pre-therapy SK-N-BE(1)	128,132,135,137
BE(2)-M17	M	24 mo	4	Bone marrow	CT/RT	N	Amplified	Loss	Normal	Gain	Parental SK-N-BE(2)	65,66,130
SMS-KCN	M	11 mo	4	Adrenal gland	None	N	Amplified	Loss	–	Gain	Post-therapy SMS-KCNR	65,128,132
IMR-32	M	13 mo	–	Abdominal mass	None	N	Amplified	Loss	Normal	–	–	65,132,133,138
SK-N-FI	M	11 y	4	Bone marrow	CT	N	Non-amplified	Normal	Normal	Normal	–	34,139–141
SH-EP	F	4 y	4	Bone marrow	CT/RT	S	Non-amplified	Normal	Normal	Gain	Parental SK-N-SH	66,68,87
SK-N-AS	F	8 y	4	Bone marrow	-	S	Non-amplified	Loss		Gain	–	130,139,141,142
SK-N-MC	F	14 y	4	Supra-orbital lymph node	CT/RT	S	Amplified	Loss		Normal	Subline SK-N-MC-IXC	132,139,142
CHLA-20	F	24 mo	4	Bone marrow	CT	–	Non-amplified	–	–	Normal	Pre-therapy CHLA-15	128,131,133
NB1643	–	36 mo	4	Retroperitoneal mass	None	–	Amplified	Loss	–	Normal	–	140,143
NB19	F	12 mo	4	Bone marrow	CT	–	Amplified	Loss	–	–	–	130,132

Note: BM: bone marrow; AG: adrenal gland; INSS: International Neuroblastoma Staging System; AM: abdominal mass; RM: retroperitoneal mass; LN: lymph node; CT: chemotherapy; RT: radiotherapy; AMN: amplified MYCN; NAMN: non-amplified MYCN; (–): non determined.

for growth factors and cytokines.[89] Tumors of epithelial origin express CD44 in multiple isoforms, which has been associated with a role in regulating the EMT process and in conferring adaptive plasticity to cancer cells.[90,91] In NB, CD44 expression has been correlated with a favorable prognosis, being reported with low or absent expression in advanced stage tumors.[92]

The high expression of ECM components also characterizes this cell phenotype.[66] Fibronectin is a secreted glycoprotein that is found in a soluble form circulating in the plasma, but also in an insoluble fibrillar form in the ECM, acting as a critical substrate for cell adhesion and migration.[93] In cancer cells, dysregulation of fibronectin cell adhesion and migration has been implicated in tumor development and metastasis, pointing out fibronectin as a cancer biomarker of EMT reversion and substrate-adherence cellular differentiation.[94,95]

Vimentin, one of the cytoskeleton main intermediate filaments, is present in normal mesenchymal cells and is responsible for maintaining the cellular integrity, stress resistance, and the migration of cancer cells that have undergone EMT.[96,97] This protein is initially expressed by early neuronal precursors and is essential for neuritogenesis, as it is gradually replaced by neurofilaments.[98] Immature and motile glial cells also produce vimentin, whereas mature glial cells cease its expression and begin to express the glial fibrillar acidic protein (GFAP).[99] The overexpression of vimentin has been reported in many cancer types, in association with cell growth and invasion, reinforcing its role in cancer cells as a marker of the EMT process.[100] In NB, vimentin is mainly expressed in S-type cells, although it can also be expressed in I-type cells.[66,67] As the S-type cells do not express GFAP, it was suggested that they represent an embryonic neural crest precursor that may undergo terminal differentiation toward a glial, meningeal, or melanocytic phenotype.[59]

I-type cells

I-type cells, firstly described as an intermediate phenotype, have been recently described as a progenitor of the N- and S-type cells, capable of self-renewal and bidirectional differentiation.[66] I-type cells present an intermediate morphology and mixed properties of N- and S-type cells, being flat-shaped with prominent nuclei and moderate amounts of cytoplasm, possibly presenting neurofilaments.[67,101] Differently than the S-type cells, which attach strongly to the underlying substrate and do not mound, or the N-type cells, which attach weakly to the substrate, the I-type cells adhere both to the substrate and to each other, mounding and forming multiple layers of cells.[69]

Seven different antigens have been tested positively as specific markers to identify I-type cells: GPRC5C, LNGFR, TRKB, NOTCH1, PIGF2, CD133, and CD117.[65,66,102] CD133 is a transmembrane glycoprotein associated to progenitor cells and to tissue regeneration and cell differentiation processes in normal tissues. It was first described as a marker of primitive hematopoietic and neural stem cells,[103,104] and it has been used extensively as a marker of stem-like cells in NB.[105–107] CD133 is over-expressed in several NB cell lines,

where it acts to repress neural differentiation and elongation, accelerating cell proliferation and colony formation.[108] The investigation of the clinical significance of CD133 expression in NB tumors has shown its association to a poor outcome[109] and chemoresistance.[110–112]

CD117, also called c-KIT, is another marker mostly used to characterize I-type cells in NB. It is a tyrosine kinase receptor of the class III subfamily.[113] The binding of its ligand, stem cell factor (SCF), and SCF/c-KIT signaling contributes to both tissue development and homeostasis, including the maintenance of the stemness status of precursor cells in several adult tissues.[114] In NB, it was described as a marker of tumor-initiating cells, involved in cell growth and differentiation,[101] clonogenic capacity, and drug resistance.[115] c-KIT expression in NB cell lines was found to be induced under hypoxic conditions,[116] which suggests that c-KIT+ cells represent an aggressive entity of NB, whose growth may be influenced by the tumor microenvironment.

Genetic regulators of NB phenotypes

Transcriptional plasticity in cancer cells can control cellular fates by regulation of transcriptional factors that can bind to promoters, enhancers, and super-enhancers, thus forming interconnected auto-regulatory loops that constitute the core regulatory circuitry (CRC).[117] Distinct CRCs have also been shown to identify cell lineage phenotypes in NB. Boeva et al. identified three different identities in NB cell lineages[118]: the non-neuronal neural crest cells phenotype, driven by a CRC module containing the AP-1 transcription factor; the sympathetic noradrenergic phenotype, defined by a CReC module that includes the PHOX2B, HAND2, and GATA3 transcription factors, and a third phenotype consisting of a mix of both CRCs. Another profile was defined by Groningen *et al.*,[119] which named two subsets of phenotypes in NB: the adrenergic lineage (ADRN), characterized by the CRC, including PHOX2a, PHOX2b, and DBH transcription factors; and the mesenchymal lineage (MES), characterized by the CRC that includes the SNAI2, VIM, and FN1 transcription factors. Later, the MES and ADRN populations were shown to be able to transdifferentiate into one another by a feedforward loop controlled by the NOTCH signaling pathway.[61]

MicroRNAs (miRNAs) can also differ in NB phenotypes. miRNAs are a class of non-coding endogenous RNA molecules that have been identified to play a critical role in cancer, regulating several target genes associated with aggressive tumor phenotypes, such as the ones that promote metastatic development and treatment resistance. In NB, the expression of miR-21, miR-221, and miR-335 was exclusively observed in non-neuronal cells, while the expression of miR-124 and miR-375 was only observed in neuroblastic cells.[86] The regulatory role of miR-335 in non-neuronal cells was described in the modulation of the expression levels of the neural differentiation modulators HAND1 and JAG1,[86] and in the suppression of cell invasion and metastasis by regulation of the TGF-β pathway.[120] Global miRNAs expression profiles were also shown to be distinct in NB tumors with diverse prognosis.[121,122]

The most used NB cell lines as in vitro models

Cancer-derived cell lines have been widely used for research and proven to be useful models to understand the multiple and diverse processes in cancer, as well as for discovering and evaluating the efficacy of treatment; their unique molecular and phenotype characteristics should be taken into consideration when choosing the most appropriate one as an *in vitro* model.

During the preparation of this manuscript, the PubMed database was searched for human NB cell lines citations from the last 10 years. Sixteen cell lines were found (some of them sublines) that were cited at least five times on this defined period (Table 2). All of them were isolated from high-risk NB patients, classified as being in stage 4, according to the International Neuroblastoma Staging System,[123] except for cell line IMR-32, which was derived from a patient with an unknown stage. Most of the cell lines are derived from bone marrow metastases from patients that underwent several lines of treatment.

The SH-SY5Y cell line and its parental line, SK-N-SH, represent the most frequently cited NB cell lines, found over 5000 times in the mentioned searched period. SK-N-SH represents an I-type cell line used to subclone a few different cell lines in 1978 by Biedler, including SH-SY5Y and SH-EP. In the above study, neuroblast-like cells from SK-N-SH were subcloned using metal cylinders isolation and single-cell culture. The first clone was called SH-SY, which was further subcloned into the SH-SY5, and finally into SH-SH5Y. Similarly, the epithelial-like cells from the SK-N-SH were also subcloned into SH-EP and SH-FE.[124] Therefore, the SH-SH5Y is an N-type cell line, although some studies demonstrate that it is also composed of S-type cells,[67] while the SH-EP represents a predominant S-type lineage. Both of these cell lines, however, present the same profile of genetic alterations, with no amplification of *MYCN* and no chromosome abnormalities on 1p and 11q, but with gains on 17q.[42,125,126] The SH-SY5Y and SK-N-SH are extensively used for the study of neuronal diseases,[127] as they can be differentiated into a more mature neuron-like phenotype with neuroblast-like morphology that is characterized by neuronal markers like tyrosine hydroxylase (TH) and DβH, characteristics of catecholaminergic neurons.[60]

Contrary to the above described NB cell lines, others were collected at diagnosis, before any treatment. The IMR-32 and SMS-KCN represent the most frequently used ones; both are N-type cells with *MYCN* amplification and functional p53, along with the loss of chromosome 1p.[125,128,129] The SMS-KCN/SMS-KCNR, CHLA-15/CHLA-20, and SK-N-BE(1)/SK-N-BE(2) are paired cell lines, each pair obtained from the same patient before and after treatment (Childhood Cancer Repository – accessed at www.cccells.org/cellreqs-nbl), valuable for evaluating the effects of treatment agents at baseline and at different stages of treatment.

Almost 20 years ago, Thiele published a comprehensive review with an extensive data collection of over 100 NB cell lines.[130] Those are listed in tables based on the clinical features of patients from whom they have been derived, along

with their chromosome and specific genetic alterations, biologic characteristics, and the effects of differentiation agents. In the same year, Keshelava et al. analyzed the drug resistance of 17 NB cell lines established from patients at different therapy stages.[131]

Future perspectives

Extraordinary advances were and are still obtained in basic and clinical cancer research with the use of "conventional" NB cell lines, established directly from patient cells immortalized *in vitro*. The use of these models provides the understanding of several phases of the tumorigenic process, including tumor progression and therapeutic resistance. Limitations, including the inability to reflect the natural biology of the patients' tumor and the genetic intra- and interpatient heterogeneity, exist. These limitations can be overcome or reduced by the use of immortalization methods of specimens directly obtained from the cancer patients that reflect their *in vivo* tumor behavior and intrinsic molecular characteristics. One of these methods includes the conditional reprogrammed cells (CRCs) method, which allows the cells to be isolated directly from patients to surpass senescence when cultivated in the presence of irradiated mouse fibroblasts and the compound Y-27632, an inhibitor of the enzyme kinase Rho.[144,145] This technology has been used in the study of pancreas, prostate, lung, and bladder cancers.[146,147] A study conducted by Mahajan *et al*., in breast tumors, has shown that CRCs maintain their original cellular and genomic signatures when compared to their original cells.[148] More recently, CRCs were also established from murine NB cell lines from TH-MYCN mice, which were shown to preserve the cellular heterogeneity observed in *in vivo*.[149]

Induced pluripotent human stem cells (hiPSCs) are based on the reprograming of differentiated adult cells of somatic origin that reach the stage of cell undifferentiation and pluripotency *in vitro*.[150,151] Due to the plasticity of the hiPSCs regarding the differentiation into various types of cells and tissues, they are widely used for the reconstruction of tissues or organs of patients with a variety of diseases. In cancer, as the CRC cells, they can be widely used to study several tumors phenotypes in a patient-specific model.[152,153]

Patient-derived tumor xenografts, which are generated by transplanting fresh tumor tissues from patients into immunodeficient mice, can retain tumor heterogeneity and genomic stability, as well as reproduce complex cancer–stroma and cancer–matrix interactions, better predicting drug responses.[154,155] However, their application in medicine can be limited, due to its high cost and long processing time.[155,156] More recently, patient derived tumor organoids (PDTO) have been promising superior models, as they comprehend three-dimensional *in vitro* cellular structures derived from cancer stem cells that present self-renewal and self-organization capabilities, while also retaining the characteristics of their source tumor.[156,157] Beyond the lower cost and processing time, PDTOs enable easily accessible therapeutic effects in both health and tumoral organoids from the same patient, as well as CRISPR-Cas9 approaches for identification of key driver mutations.[158] Protocols to produce PDTOs from different pediatric solid tumors, including NBs, have already been developed and showed to be efficient for maintaining gene-expression profiles and epigenomes from tumors.[159] NB heterogeneities, such as phenotype markers and chromosomal aberrations, were also retained in PDTOs.[160]

As these technologies of cancer organoids and tumor cells reprogramming evolve, enabling and facilitating their routine use in laboratories is necessary, as they will become essential tools for the generation of patient-derived cell lines that can subsequently be used for the discovery of novel predictive progression markers, and drug-screening toward personalized-medicine strategies.

Conclusions

One of the most critical and current challenge in NB is the treatment of high-risk patients, whose mortality rates have not decreased in the last four decades. The high rate of treatment failure is largely due to the high degree of cellular and genetic heterogeneity of these tumors, which leads to diverse mechanisms of treatment resistance. Although the current models of NB immortalized cell lines present limitations, they are still fundamental and valuable in vitro models for the understanding of such mechanisms. Their cell lineage diversity allows to address a variety of clinical processes, such as the response to differentiation-inducing agents and cytotoxic drugs, and the rapid progression from a primary tumor to metastasis. The availability and the choice of the most appropriate NB cell line, or NB cell line panel, based on its distinct cellular and genetic phenotypic profile is of utmost importance for clinical research, which may be complemented by the generation of NB reprogrammed tumor cells.

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REFERENCES

1. Schleiermacher G, Janoueix-Lerosey I, Delattre O. Recent insights into the biology of neuroblastoma. *Int J Cancer* 2014;**135**:2249–61
2. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, Weiss WA. Neuroblastoma. *Nat Rev Dis Primers* 2016;**2**:16078
3. Brodeur GM. Spontaneous regression of neuroblastoma. *Cell Tissue Res* 2018;**372**:277–86
4. Johnsen JI, Dyberg C, Wickström M. Neuroblastoma-A neural crest derived embryonal malignancy. *Front Mol Neurosci* 2019;**12**:9
5. Morgenstern DA, London WB, Stephens D, Volchenboum SL, Simon T, Nakagawara A, Shimada H, Schleiermacher G, Matthay KK, Cohn SL, Pearson AD, Irwin MS. Prognostic significance of pattern and burden of metastatic disease in patients with stage 4 neuroblastoma: a study from the international neuroblastoma risk group database. *Eur J Cancer* 2016;**65**:1–10
6. Shimada H, Umehara S, Monobe Y, Hachitanda Y, Nakagawa A, Goto S, Gerbing RB, Stram DO, Lukens JN, Matthay KK. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors: a report from the children's cancer group. *Cancer* 2001;**92**:2451–61
7. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, Stram DO, Gerbing RB, Lukens JN, Matthay KK, Castleberry RP. The international neuroblastoma pathology classification (the Shimada system). *Cancer* 1999;**86**:364–72
8. Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and treatment. *Cell Tissue Res* 2018;**372**:195–209
9. Brodeur GM, Minturn JE, Ho R, Simpson AM, Iyer R, Varela CR, Light JE, Kolla V, Evans AE. Trk receptor expression and inhibition in neuroblastomas. *Clin Cancer Res* 2009;**15**:3244–50
10. Iyer R, Varela CR, Minturn JE, Ho R, Simpson AM, Light JE, Evans AE, Zhao H, Thress K, Brown JL, Brodeur GM. AZ64 inhibits TrkB and enhances the efficacy of chemotherapy and local radiation in neuroblastoma xenografts. *Cancer Chemother Pharmacol* 2012;**70**:477–86
11. Meldolesi J. Neurotrophin Trk receptors: new targets for cancer therapy. *Rev Physiol Biochem Pharmacol* 2018;**174**:67–79
12. Li Z, Zhang Y, Tong Y, Tong J, Thiele CJ. Trk inhibitor attenuates the BDNF/TrkB-induced protection of neuroblastoma cells from etoposide in vitro and in vivo. *Cancer Biol Ther* 2015;**16**:477–83
13. Aygun N. Biological and genetic features of neuroblastoma and their clinical importance. *Curr Pediatr Rev* 2018;**14**:73–90
14. Cao Y, Jin Y, Yu J, Wang J, Yan J, Zhao Q. Research progress of neuroblastoma related gene variations. *Oncotarget* 2017;**8**:18444–55
15. Kamihara J, Bourdeaut F, Foulkes WD, Molenaar JJ, Mossé YP, Nakagawara A, Parareda A, Scollon SR, Schneider KW, Skalet AH, States LJ, Walsh MF, Diller LR, Brodeur GM. Retinoblastoma and neuroblastoma predisposition and surveillance. *Clin Cancer Res* 2017;**23**:e98–e106
16. Trigg RM, Turner SD. ALK in neuroblastoma: biological and therapeutic implications. *Cancers* 2018;**10**:113
17. Deyell RJ, Attiyeh EF. Advances in the understanding of constitutional and somatic genomic alterations in neuroblastoma. *Cancer Genet* 2011;**204**:113–21
18. Masecchia S, Coco S, Barla A, Verri A, Tonini GP. Genome instability model of metastatic neuroblastoma tumorigenesis by a dictionary learning algorithm. *BMC Med Genomics* 2015;**8**:57
19. Thorner Aloop PS The molecular genetic profile of neuroblastoma. *Diagn Histopathol* 2014;**20**:76–83
20. Uryu K, Nishimura R, Kataoka K, Sato Y, Nakazawa A, Suzuki H, Yoshida K, Seki M, Hiwatari M, Isobe T, Shiraishi Y, Chiba K, Tanaka H, Miyano S, Koh K, Hanada R, Oka A, Hayashi Y, Ohira M, Kamijo T, Nagase H, Takimoto T, Tajiri T, Nakagawara A, Ogawa S, Takita J. Identification of the genetic and clinical characteristics of neuroblastomas using genome-wide analysis. *Oncotarget* 2017;**8**:107513–29
21. Ho N, Peng H, Mayoh C, Liu PY, Atmadibrata B, Marshall GM, Li J, Liu T. Delineation of the frequency and boundary of chromosomal copy number variations in paediatric neuroblastoma. *Cell Cycle* 2018;**17**:749–58
22. Berbegall AP, Villamón E, Piqueras M, Tadeo I, Djos A, Ambros PF, Martinsson T, Ambros IM, Cañete A, Castel V, Navarro S, Noguera R. Comparative genetic study of intratumoral heterogenous MYCN amplified neuroblastoma versus aggressive genetic profile neuroblastic tumors. *Oncogene* 2016;**35**:1423–32
23. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984;**224**:1121–4
24. Attiyeh EF, London WB, Mossé YP, Wang Q, Winter C, Khazi D, McGrady PW, Seeger RC, Look AT, Shimada H, Brodeur GM, Cohn SL, Matthay KK, Maris JM; Group CsO. Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med* 2005;**353**:2243–53
25. Stafman LL, Beierle EA. Cell proliferation in neuroblastoma. *Cancers* 2016;**8**:13
26. Slack A, Chen Z, Tonelli R, Pule M, Hunt L, Pession A, Shohet JM. The p53 regulatory gene MDM2 is a direct transcriptional target of MYCN in neuroblastoma. *Proc Natl Acad Sci USA* 2005;**102**:731–6
27. Van Maerken T, Rihani A, Dreidax D, De Clercq S, Yigit N, Marine JC, Westermann F, De Paepe A, Vandesompele J, Speleman F. Functional analysis of the p53 pathway in neuroblastoma cells using the small-molecule MDM2 antagonist nutlin-3. *Mol Cancer Ther* 2011;**10**:983–93
28. Liu Z, Thiele CJ. ALK and MYCN: when two oncogenes are better than one. *Cancer Cell* 2012;**21**:325–6
29. Yang L, Li Y, Wei Z, Chang X. Coexpression network analysis identifies transcriptional modules associated with genomic alterations in neuroblastoma. *Biochim Biophys Acta Mol Basis Dis* 2018;**1864**:2341–8
30. Henrich KO, Schwab M, Westermann F. 1p36 tumor suppression – a matter of dosage? *Cancer Res* 2012;**72**:6079–88
31. Bagchi A, Papazoglu C, Wu Y, Capurso D, Brodt M, Francis D, Bredel M, Vogel H, Mills AA. CHD5 is a tumor suppressor at human 1p36. *Cell* 2007;**128**:459–75
32. Carén H, Fransson S, Ejeskär K, Kogner P, Martinsson T. Genetic and epigenetic changes in the common 1p36 deletion in neuroblastoma tumours. *Br J Cancer* 2007;**97**:1416–24
33. Okawa ER, Gotoh T, Manne J, Igarashi J, Fujita T, Silverman KA, Xhao H, Mosse YP, White PS, Brodeur GM. Expression and sequence analysis of candidates for the 1p36.31 tumor suppressor gene deleted in neuroblastomas. *Oncogene* 2008;**27**:803–10
34. Fujita T, Igarashi J, Okawa ER, Gotoh T, Manne J, Kolla V, Kim J, Zhao H, Pawel BR, London WB, Maris JM, White PS, Brodeur GM. CHD5, a tumor suppressor gene deleted from 1p36.31 in neuroblastomas. *J Natl Cancer Inst* 2008;**100**:940–9
35. Koyama H, Zhuang T, Light JE, Kolla V, Higashi M, McGrady PW, London WB, Brodeur GM. Mechanisms of CHD5 inactivation in neuroblastomas. *Clin Cancer Res* 2012;**18**:1588–97
36. Thompson PM, Gotoh T, Kok M, White PS, Brodeur GM. CHD5, a new member of the chromodomain gene family, is preferentially expressed in the nervous system. *Oncogene* 2003;**22**:1002–11
37. Kolla V, Zhuang T, Higashi M, Naraparaju K, Brodeur GM. Role of CHD5 in human cancers: 10 years later. *Cancer Res* 2014;**74**:652–8
38. Higashi M, Kolla V, Iyer R, Naraparaju K, Zhuang T, Kolla S, Brodeur GM. Retinoic acid-induced CHD5 upregulation and neuronal differentiation of neuroblastoma. *Mol Cancer* 2015;**14**:150
39. Craig BT, Rellinger EJ, Alvarez AL, Dusek HL, Qiao J, Chung DH. Induced differentiation inhibits sphere formation in neuroblastoma. *Biochem Biophys Res Commun* 2016;**477**:255–9

40. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM, Gerbing RB, Reynolds CP. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's cancer group. *N Engl J Med* 1999;**341**:1165–73
41. Ambros PF, Ambros IM, Brodeur GM, Haber M, Khan J, Nakagawara A, Schleiermacher G, Speleman F, Spitz R, London WB, Cohn SL, Pearson AD, Maris JM. International consensus for neuroblastoma molecular diagnostics: report from the international neuroblastoma risk group (INRG) biology committee. *Br J Cancer* 2009;**100**:1471–82
42. Carén H, Kryh H, Nethander M, Sjöberg RM, Träger C, Nilsson S, Abrahamsson J, Kogner P, Martinsson T. High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. *Proc Natl Acad Sci USA* 2010;**107**:4323–8
43. Takagi M, Yoshida M, Nemoto Y, Tamaichi H, Tsuchida R, Seki M, Uryu K, Nishii R, Miyamoto S, Saito M, Hanada R, Kaneko H, Miyano S, Kataoka K, Yoshida K, Ohira M, Hayashi Y, Nakagawara A, Ogawa S, Mizutani S, Takita J. Loss of DNA damage response in neuroblastoma and utility of a PARP inhibitor. *J Natl Cancer Inst* 2017;**109**:djx062
44. Mlakar V, Jurkovic Mlakar S, Lopez G, Maris JM, Ansari M, Gumy-Pause F. 11q deletion in neuroblastoma: a review of biological and clinical implications. *Mol Cancer* 2017;**16**:114
45. Ribelles AJ, Barbera S, Yanez Y, Gargallo P, Segura V, Juan B, Noguera R, Piqueras M, Fornes-Ferrer V, de Mora JF, Canete A, Castel V. Clinical features of neuroblastoma with 11q deletion: an increase in relapse probabilities in localized and 4S stages. *Sci Rep* 2019;**9**:13806
46. Michels E, Hoebeeck J, De Preter K, Schramm A, Brichard B, De Paepe A, Eggert A, Laureys G, Vandesompele J, Speleman F. CADM1 is a strong neuroblastoma candidate gene that maps within a 3.72 Mb critical region of loss on 11q23. *BMC Cancer* 2008;**8**:173
47. Hoebeeck J, Michels E, Pattyn F, Combaret V, Vermeulen J, Yigit N, Hoyoux C, Laureys G, De Paepe A, Speleman F, Vandesompele J. Aberrant methylation of candidate tumor suppressor genes in neuroblastoma. *Cancer Lett* 2009;**273**:336–46
48. Murakami Y. Involvement of a cell adhesion molecule, TSLC1/IGSF4, in human oncogenesis. *Cancer Sci* 2005;**96**:543–52
49. Hartsough EJ, Weiss MB, Heilman SA, Purwin TJ, Kugel CH 3rd, Rosenbaum SR, Erkes DA, Tiago M, HooKim K, Chervoneva I, Aplin AE. CADM1 is a TWIST1-regulated suppressor of invasion and survival. *Cell Death Dis* 2019;**10**:281
50. Gumy-Pause F, Pardo B, Khoshbeen-Boudal M, Ansari M, Gayet-Ageron A, Sappino AP, Attiyeh EF, Ozsahin H. GSTP1 hypermethylation is associated with reduced protein expression, aggressive disease and prognosis in neuroblastoma. *Genes Chromosomes Cancer* 2012;**51**:174–85
51. Nowacki S, Skowron M, Oberthuer A, Fagin A, Voth H, Brors B, Westermann F, Eggert A, Hero B, Berthold F, Fischer M. Expression of the tumour suppressor gene CADM1 is associated with favourable outcome and inhibits cell survival in neuroblastoma. *Oncogene* 2008;**27**:3329–38
52. Combaret V, Brejon S, Iacono I, Schleiermacher G, Pierron G, Ribeiro A, Bergeron C, Marabelle A, Puisieux A. Determination of 17q gain in patients with neuroblastoma by analysis of circulating DNA. *Pediatr Blood Cancer* 2011;**56**:757–61
53. Kuzyk A, Gartner J, Mai S. Identification of neuroblastoma subgroups based on three-dimensional telomere organization. *Transl Oncol* 2016;**9**:348–56
54. Godfried MB, Veenstra M, V Sluis P, Boon K, V Asperen R, Hermus MC, V Schaik BD, Voûte TP, Schwab M, Versteeg R, Caron HN. The N-myc and c-myc downstream pathways include the chromosome 17q genes nm23-H1 and nm23-H2. *Oncogene* 2002;**21**:2097–101
55. Krona C, Carén H, Sjöberg RM, Sandstedt B, Laureys G, Kogner P, Martinsson T. Analysis of neuroblastoma tumour progression; loss of PHOX2B on 4p13 and 17q gain are early events in neuroblastoma tumourigenesis. *Int J Oncol* 2008;**32**:575–83
56. Murray MR, Stout AP. Distinctive characteristics of the sympathicoblastoma cultivated in vitro: a method for prompt diagnosis. *Am J Pathol* 1947;**23**:429–41
57. Chakrabarti L, Abou-Antoun T, Vukmanovic S, Sandler AD. Reversible adaptive plasticity: a mechanism for neuroblastoma cell heterogeneity and chemo-resistance. *Front Oncol* 2012;**2**:82
58. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018;**15**:81–94
59. Ciccarone V, Spengler BA, Meyers MB, Biedler JL, Ross RA. Phenotypic diversification in human neuroblastoma cells: expression of distinct neural crest lineages. *Cancer Res* 1989;**49**:219–25
60. Ross RA, Spengler BA, Biedler JL. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J Natl Cancer Inst* 1983;**71**:741–7
61. van Groningen T, Akogul N, Westerhout EM, Chan A, Hasselt NE, Zwijnenburg DA, Broekmans M, Stroeken P, Haneveld F, Hooijer GKJ, Savci-Heijink CD, Lakeman A, Volckmann R, van Sluis P, Valentijn LJ, Koster J, Versteeg R, van Nes J. A NOTCH feed-forward loop drives reprogramming from adrenergic to mesenchymal state in neuroblastoma. *Nat Commun* 2019;**10**:1530
62. Cohen N, Betts DR, Rechavi G, Amariglio N, Trakhtenbrot L. Clonal expansion and not cell interconversion is the basis for the neuroblast and nonneuronal types of the SK-N-SH neuroblastoma cell line. *Cancer Genet Cytogenet* 2003;**143**:80–4
63. Goranov BB, Redfern CP. Cytogenetic differences and neuroblastoma cell interconversion. *Cancer Genet Cytogenet* 2004;**149**:173–4
64. Cimmino F, Pezone L, Avitabile M, Acierno G, Andolfo I, Capasso M, Iolascon A. Inhibition of hypoxia inducible factors combined with all-trans retinoic acid treatment enhances glial transdifferentiation of neuroblastoma cells. *Sci Rep* 2015;**5**:11158
65. Walton JD, Kattan DR, Thomas SK, Spengler BA, Guo HF, Biedler JL, Cheung NK, Ross RA. Characteristics of stem cells from human neuroblastoma cell lines and in tumors. *Neoplasia* 2004;**6**:838–45
66. Ross RA, Walton JD, Han D, Guo HF, Cheung NK. A distinct gene expression signature characterizes human neuroblastoma cancer stem cells. *Stem Cell Res* 2015;**15**:419–26
67. Bell N, Hann V, Redfern CP, Cheek TR. Store-operated Ca(2+) entry in proliferating and retinoic acid-differentiated N- and S-type neuroblastoma cells. *Biochim Biophys Acta* 2013;**1833**:643–51
68. Corey JM, Gertz CC, Sutton TJ, Chen Q, Mycek KB, Wang BS, Martin AA, Johnson SL, Feldman EL. Patterning N-type and S-type neuroblastoma cells with pluronic F108 and ECM proteins. *J Biomed Mater Res A* 2010;**93**:673–86
69. Ross RA, Biedler JL, Spengler BA. A role for distinct cell types in determining malignancy in human neuroblastoma cell lines and tumors. *Cancer Lett* 2003;**197**:35–9
70. Whitworth CL, Redfern CPF, Cheek TR. Differentiation-induced remodelling of store-operated calcium entry is independent of neuronal or glial phenotype but modulated by cellular context. *Mol Neurobiol* 2019;**56**:857–72
71. Goodson HV, Jonasson EM. Microtubules and microtubule-associated proteins. *Cold Spring Harb Perspect Biol* 2018;**10**:a022608
72. Hagenbuchner J, Lungkofler L, Kiechl-Kohlendorfer U, Viola G, Ferlin MG, Ausserlechner MJ, Obexer P. The tubulin inhibitor MG-2477 induces autophagy-regulated cell death, ROS accumulation and activation of FOXO3 in neuroblastoma. *Oncotarget* 2017;**8**:32009–26
73. Parker AL, Kavallaris M, McCarroll JA. Microtubules and their role in cellular stress in cancer. *Front Oncol* 2014;**4**:153
74. Don S, Verrills NM, Liaw TY, Liu ML, Norris MD, Haber M, Kavallaris M. Neuronal-associated microtubule proteins class III beta-tubulin and MAP2c in neuroblastoma: role in resistance to microtubule-targeted drugs. *Mol Cancer Ther* 2004;**3**:1137–46
75. Liu J, Li Y. Upregulation of MAPK10, TUBB2B and RASL11B may contribute to the development of neuroblastoma. *Mol Med Rep* 2019;**20**:3475–86
76. Boumil EF, Vohnoutka R, Lee S, Pant H, Shea TB. Assembly and turnover of neurofilaments in growing axonal neurites. *Biol Open* 2018;**7**: bio028795

77. Ho CL, Liem RK. Intermediate filaments in the nervous system: implications in cancer. *Cancer Metastasis Rev* 1996;**15**:483–97
78. Pal HA, Singh A, Sheikh PA, Panjla A, Kumar A, Verma S. Peptide-based scaffold for nitric oxide induced differentiation of neuroblastoma cells. *Chembiochem* 2018;**19**:1127–31
79. Calmon MF, Jeschke J, Zhang W, Dhir M, Siebenkäs C, Herrera A, Tsai HC, O'Hagan HM, Pappou EP, Hooker CM, Fu T, Schuebel KE, Gabrielson E, Rahal P, Herman JG, Baylin SB, Ahuja N. Epigenetic silencing of neurofilament genes promotes an aggressive phenotype in breast cancer. *Epigenetics* 2015;**10**:622–32
80. Encinas M, Iglesias M, Liu Y, Wang H, Muhaisen A, Ceña V, Gallego C, Comella JX. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. *J Neurochem* 2000;**75**:991–1003
81. Teppola H, Sarkanen JR, Jalonen TO, Linne ML. Morphological differentiation towards neuronal phenotype of SH-SY5Y neuroblastoma cells by estradiol, retinoic acid and cholesterol. *Neurochem Res* 2016;**41**:731–47
82. Foley J, Witte D, Chiu FC, Parysek LM. Expression of the neural intermediate filament proteins peripherin and neurofilament-66/alpha-internexin in neuroblastoma. *Lab Invest* 1994;**71**:193–9
83. Kovalevich J, Langford D. Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods in Molecular Biology (Clifton, NJ)* 2013;**1078**:9–21
84. Xie HR, Hu LS, Li GY. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. *Chin Med J* 2010;**123**:1086–92
85. Cubells JF, Zabetian CP. Human genetics of plasma dopamine beta-hydroxylase activity: applications to research in psychiatry and neurology. *Psychopharmacology (Berl)* 2004;**174**:463–76
86. Samaraweera L, Grandinetti KB, Huang R, Spengler BA, Ross RA. MicroRNAs define distinct human neuroblastoma cell phenotypes and regulate their differentiation and tumorigenicity. *BMC Cancer* 2014;**14**:309
87. Meyer A, van Golen CM, Kim B, van Golen KL, Feldman EL. Integrin expression regulates neuroblastoma attachment and migration. *Neoplasia* 2004;**6**:332–42
88. Basakran NS. CD44 as a potential diagnostic tumor marker. *Saudi Med J* 2015;**36**:273–9
89. Senbanjo LT, Chellaiah MA. CD44: a multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. *Front Cell Dev Biol* 2017;**5**:18
90. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol* 2018;**11**:64
91. Wang Z, Zhao K, Hackert T, Zöller M. CD44/CD44v6 a reliable companion in cancer-initiating cell maintenance and tumor progression. *Front Cell Dev Biol* 2018;**6**:97
92. Siapati EK, Rouka E, Kyriakou D, Vassilopoulos G. Neuroblastoma cells negative for CD44 possess tumor-initiating properties. *Cell Oncol (Dordr)* 2011;**34**:189–97
93. Zollinger AJ, Smith ML. Fibronectin, the extracellular glue. *Matrix Biol* 2017;**60-61**:27–37
94. Jia D, Entersz I, Butler C, Foty RA. Fibronectin matrix-mediated cohesion suppresses invasion of prostate cancer cells. *BMC Cancer* 2012;**12**:94
95. Rick JW, Chandra A, Dalle Ore C, Nguyen AT, Yagnik G, Aghi MK. Fibronectin in malignancy: Cancer-specific alterations, protumoral effects, and therapeutic implications. *Semin Oncol* 2019;**46**:284–90
96. Battaglia RA, Delic S, Herrmann H, Snider NT. Vimentin on the move: new developments in cell migration. *F1000Res* 2018;**7**:1796
97. De Pascalis C, Perez-Gonzalez C, Seetharaman S, Boeda B, Vianay B, Burute M, Leduc C, Borghi N, Trepat X, Etienne-Manneville S. Intermediate filaments control collective migration by restricting traction forces and sustaining cell-cell contacts. *J Cell Biol* 2018;**217**:3031–44
98. Dubey M, Hoda S, Chan WK, Pimenta A, Ortiz DD, Shea TB. Reexpression of vimentin in differentiated neuroblastoma cells enhances elongation of axonal neurites. *J Neurosci Res* 2004;**78**:245–9
99. Backhovens H, Gheuens J, Slegers H. Expression of glial fibrillary acidic protein in rat C6 glioma relates to vimentin and is independent of cell-cell contact. *J Neurochem* 1987;**49**:348–54
100. Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. *Cell Mol Life Sci* 2011;**68**:3033–46
101. Qi S, Zheng J, Zhu H, Yang L, Xiao X. Identification of neuroblastoma stem cells by characterization of side population cells in the human neuroblastoma SK-N-SH cell line. *J Pediatr Surg* 2010;**45**:2305–11
102. Ross RA, Spengler BA. Human neuroblastoma stem cells. *Semin Cancer Biol* 2007;**17**:241–7
103. Li W, Lee MR, Kim T, Kim YW, Cho MY. Activated STAT3 may participate in tumor progression through increasing CD133/survivin expression in early stage of Colon cancer. *Biochem Biophys Res Commun* 2018;**497**:354–61
104. Mizrak D, Brittan M, Alison M. CD133: molecule of the moment. *J Pathol* 2008;**214**:3–9
105. Kim KW, Kim JY, Qiao J, Clark RA, Powers CM, Correa H, Chung DH. Dual-targeting AKT2 and ERK in cancer stem-like cells in neuroblastoma. *Oncotarget* 2019;**10**:5645–59
106. Schiapparelli P, Enguita-German M, Balbuena J, Rey JA, Lazcoz P, Castresana JS. Analysis of stemness gene expression and CD133 abnormal methylation in neuroblastoma cell lines. *Oncol Rep* 2010;**24**:1355–62
107. Khalil MA, Hrabeta J, Groh T, Prochazka P, Doktorova H, Eckschlager T. Valproic acid increases CD133 positive cells that show low sensitivity to cytostatics in neuroblastoma. *PLoS One* 2016;**11**:e0162916
108. Takenobu H, Shimozato O, Nakamura T, Ochiai H, Yamaguchi Y, Ohira M, Nakagawara A, Kamijo T. CD133 suppresses neuroblastoma cell differentiation via signal pathway modification. *Oncogene* 2011;**30**:97–105
109. Khalil MA, Hrabeta J, Cipro S, Stiborova M, Vicha A, Eckschlager T. Neuroblastoma stem cells – mechanisms of chemoresistance and histone deacetylase inhibitors. *Neoplasma* 2012;**59**:737–46
110. Zhong ZY, Shi BJ, Zhou H, Wang WB. CD133 expression and MYCN amplification induce chemoresistance and reduce average survival time in pediatric neuroblastoma. *J Int Med Res* 2018;**46**:1209–20
111. Baj G, Tongiorgi E. BDNF splice variants from the second promoter cluster support cell survival of differentiated neuroblastoma upon cytotoxic stress. *J Cell Sci* 2009;**122**:36–43
112. Sartelet H, Imbriglio T, Nyalendo C, Haddad E, Annabi B, Duval M, Fetni R, Victor K, Alexendrov L, Sinnett D, Fabre M, Vassal G. CD133 expression is associated with poor outcome in neuroblastoma via chemoresistance mediated by the AKT pathway. *Histopathology* 2012;**60**:1144–55
113. Pittoni P, Piconese S, Tripodo C, Colombo MP. Tumor-intrinsic and -extrinsic roles of c-Kit: mast cells as the primary off-target of tyrosine kinase inhibitors. *Oncogene* 2011;**30**:757–69
114. Hutt KJ, McLaughlin EA, Holland MK. Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. *Mol Hum Reprod* 2006;**12**:61–9
115. Lau ST, Hansford LM, Chan WK, Chan GC, Wan TS, Wong KK, Kaplan DR, Tam PK, Ngan ES. Prokineticin signaling is required for the maintenance of a de novo population of c-KIT^{+} cells to sustain neuroblastoma progression. *Oncogene* 2015;**34**:1019–34
116. Jogi A, Ora I, Nilsson H, Lindeheim A, Makino Y, Poellinger L, Axelson H, Pahlman S. Hypoxia alters gene expression in human neuroblastoma cells toward an immature and neural crest-like phenotype. *Proc Natl Acad Sci USA* 2002;**99**:7021–6
117. Saint-Andre V, Federation AJ, Lin CY, Abraham BJ, Reddy J, Lee TI, Bradner JE, Young RA. Models of human core transcriptional regulatory circuitries. *Genome Res* 2016;**26**:385–96
118. Boeva V, Louis-Brennetot C, Peltier A, Durand S, Pierre-Eugène C, Raynal V, Etchevers HC, Thomas S, Lermine A, Daudigeos-Dubus E, Geoerger B, Orth MF, Grünewald TGP, Diaz E, Ducos B, Surdez D, Carcaboso AM, Medvedeva I, Deller T, Combaret V, Lapouble E, Pierron G, Grossetête-Lalami S, Baulande S, Schleiermacher G, Barillot E, Rohrer H, Delattre O, Janoueix-Lerosey I. Heterogeneity of neuroblastoma cell identity defined by transcriptional circuitries. *Nat Genet* 2017;**49**:1408–13

119. van Groningen T, Koster J, Valentijn LJ, Zwijnenburg DA, Akogul N, Hasselt NE, Broekmans M, Haneveld F, Nowakowska NE, Bras J, van Noesel CJM, Jongejan A, van Kampen AH, Koster L, Baas F, van Dijk-Kerkhoven L, Huizer-Smit M, Lecca MC, Chan A, Lakeman A, Molenaar P, Volckmann R, Westerhout EM, Hamdi M, van Sluis PG, Ebus ME, Molenaar JJ, Tytgat GA, Westerman BA, van Nes J, Versteeg R. Neuroblastoma is composed of two super-enhancer-associated differentiation states. *Nat Genet* 2017;**49**:1261–6
120. Lynch J, Fay J, Meehan M, Bryan K, Watters KM, Murphy DM, Stallings RL. MiRNA-335 suppresses neuroblastoma cell invasiveness by direct targeting of multiple genes from the non-canonical TGF-beta signalling pathway. *Carcinogenesis* 2012;**33**:976–85
121. Chen Y, Stallings RL. Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. *Cancer Res* 2007;**67**:976–83
122. Stallings RL. MicroRNA involvement in the pathogenesis of neuroblastoma: potential for microRNA mediated therapeutics. *Curr Pharm Des* 2009;**15**:456–62
123. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003;**3**:203–16
124. Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res* 1978;**38**:3751–7
125. Breit S, Ashman K, Wilting J, Rossler J, Hatzi E, Fotsis T, Schweigerer L. The N-myc oncogene in human neuroblastoma cells: down-regulation of an angiogenesis inhibitor identified as activin A. *Cancer Res* 2000;**60**:4596–601
126. Yusuf M, Leung K, Morris KJ, Volpi EV. Comprehensive cytogenomic profile of the in vitro neuronal model SH-SY5Y. *Neurogenetics* 2013;**14**:63–70
127. Niewiarowska-Sendo A, Patrzalek K, Kozik A, Guevara-Lora I. The effect of differentiation agents on inflammatory and oxidative responses of the human neuroblastoma cell line SK-N-SH. *Acta Biochim Pol* 2015;**62**:435–43
128. Keshelava N, Tsao-Wei D, Reynolds CP. Pyrazoloacridine is active in multidrug-resistant neuroblastoma cell lines with nonfunctional p53. *Clin Cancer Res* 2003;**9**:3492–502
129. Kryh H, Caren H, Erichsen J, Sjoberg RM, Abrahamsson J, Kogner P, Martinsson T. Comprehensive SNP array study of frequently used neuroblastoma cell lines; copy neutral loss of heterozygosity is common in the cell lines but uncommon in primary tumors. *BMC Genomics* 2011;**12**:443
130. Thiele CJ. Neuroblastoma cell lines. *Human Cell Culture* 1998;**1**:32
131. Keshelava N, Seeger RC, Groshen S, Reynolds CP. Drug resistance patterns of human neuroblastoma cell lines derived from patients at different phases of therapy. *Cancer Res* 1998;**58**:5396–405
132. Gilbert F, Feder M, Balaban G, Brangman D, Lurie DK, Podolsky R, Rinaldt V, Vinikoor N, Weisband J. Human neuroblastomas and abnormalities of chromosomes 1 and 17. *Cancer Res* 1984;**44**:5444–9
133. Mahller YY, Williams JP, Baird WH, Mitton B, Grossheim J, Saeki Y, Cancelas JA, Ratner N, Cripe TP. Neuroblastoma cell lines contain pluripotent tumor initiating cells that are susceptible to a targeted oncolytic virus. *PLoS One* 2009;**4**:e4235
134. Schleiermacher G, Janoueix-Lerosey I, Combaret V, Derré J, Couturier J, Aurias A, Delattre O. Combined 24-color karyotyping and comparative genomic hybridization analysis indicates predominant rearrangements of early replicating chromosome regions in neuroblastoma. *Cancer Genet Cytogenet* 2003;**141**:32–42
135. Carr J, Bell E, Pearson AD, Kees UR, Beris H, Lunec J, Tweddle DA. Increased frequency of aberrations in the p53/MDM2/p14(ARF) pathway in neuroblastoma cell lines established at relapse. *Cancer Res* 2006;**66**:2138–45
136. Kaneko T, Okita H, Nakajima H, Iijima K, Ogasawara N, Miyagawa Y, Katagiri YU, Nakagawa A, Kiyokawa N, Sato T, Fujimoto J. Neuroblastoma cells can be classified according to glycosphingolipid expression profiles identified by liquid chromatography-tandem mass spectrometry. *Int J Oncol* 2010;**37**:1279–88
137. Crawford SE, Stellmach V, Ranalli M, Huang X, Huang L, Volpert O, De Vries GH, Abramson LP, Bouck N. Pigment epithelium-derived factor (PEDF) in neuroblastoma: a multifunctional mediator of Schwann cell antitumor activity. *J Cell Sci* 2001;**114**:4421–8
138. Brodeur GM, Sekhon G, Goldstein MN. Chromosomal aberrations in human neuroblastomas. *Cancer* 1977;**40**:2256–63
139. Voigt A, Hartmann P, Zintl F. Differentiation, proliferation and adhesion of human neuroblastoma cells after treatment with retinoic acid. *Cell Adhes Commun* 2000;**7**:423–40
140. Harenza JL, Diamond MA, Adams RN, Song MM, Davidson HL, Hart LS, Dent MH, Fortina P, Reynolds CP, Maris JM. Transcriptomic profiling of 39 commonly-used neuroblastoma cell lines. *Sci Data* 2017;**4**:170033
141. Sanmartín E, Muñoz L, Piqueras M, Sirerol JA, Berlanga P, Cañete A, Castel V, Font de Mora J. Deletion of 11q in neuroblastomas drives sensitivity to PARP inhibition. *Clin Cancer Res* 2017;**23**:6875–87
142. Gogolin S, Ehemann V, Becker G, Brueckner LM, Dreidax D, Bannert S, Nolte I, Savelyeva L, Bell E, Westermann F. CDK4 inhibition restores G(1)-S arrest in MYCN-amplified neuroblastoma cells in the context of doxorubicin-induced DNA damage. *Cell Cycle* 2013;**12**:1091–104
143. Middlemas DS, Stewart CF, Kirstein MN, Poquette C, Friedman HS, Houghton PJ, Brent TP. Biochemical correlates of temozolomide sensitivity in pediatric solid tumor xenograft models. *Clin Cancer Res* 2000;**6**:998–1007
144. Dakic A, DiVito K, Fang S, Suprynowicz F, Gaur A, Li X, Palechor-Ceron N, Simic V, Choudhury S, Yu S, Simbulan-Rosenthal CM, Rosenthal D, Schlegel R, Liu X. ROCK inhibitor reduces myc-induced apoptosis and mediates immortalization of human keratinocytes. *Oncotarget* 2016;**7**:66740–53
145. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA, Nealon C, Dakic A, Simic V, Haddad BR, Rhim JS, Dritschilo A, Riegel A, McBride A, Schlegel R. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol* 2012;**180**:599–607
146. Liu X, Krawczyk E, Suprynowicz FA, Palechor-Ceron N, Yuan H, Dakic A, Simic V, Zheng YL, Sripadhan P, Chen C, Lu J, Hou TW, Choudhury S, Kallakury B, Tang DG, Darling T, Thangapazham R, Timofeeva O, Dritschilo A, Randell SH, Albanese C, Agarwal S, Schlegel R. Conditional reprogramming and long-term expansion of normal and tumor cells from human biospecimens. *Nat Protoc* 2017;**12**:439–51
147. Timofeeva OA, Palechor-Ceron N, Li G, Yuan H, Krawczyk E, Zhong X, Liu G, Upadhyay G, Dakic A, Yu S, Fang S, Choudhury S, Zhang X, Ju A, Lee MS, Dan HC, Ji Y, Hou Y, Zheng YL, Albanese C, Rhim J, Schlegel R, Dritschilo A, Liu X. Conditionally reprogrammed normal and primary tumor prostate epithelial cells: a novel patient-derived cell model for studies of human prostate cancer. *Oncotarget* 2017;**8**:22741–58
148. Mahajan AS, Sugita BM, Duttargi AN, Saenz F, Krawczyk E, McCutcheon JN, Fonseca AS, Kallakury B, Pohlmann P, Gusev Y, Cavalli LR. Genomic comparison of early-passage conditionally reprogrammed breast cancer cells to their corresponding primary tumors. *PLoS One* 2017;**12**:e0186190
149. Krawczyk E, Hong SH, Galli S, Trinh E, Wietlisbach L, Misiukiewicz SF, Tilan JU, Chen YS, Schlegel R, Kitlinska J. Murine neuroblastoma cell lines developed by conditional reprogramming preserve heterogeneous phenotypes observed in vivo. *Lab Invest* 2020;**100**:38–51
150. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without myc from mouse and human fibroblasts. *Nat Biotechnol* 2008;**26**:101–6
151. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76
152. Chao HM, Chern E. Patient-derived induced pluripotent stem cells for models of cancer and cancer stem cell research. *J Formos Med Assoc* 2018;**117**:1046–57
153. Papapetrou EP. Patient-derived induced pluripotent stem cells in cancer research and precision oncology. *Nat Med* 2016;**22**:1392–401
154. Yoshida GJ. Applications of patient-derived tumor xenograft models and tumor organoids. *J Hematol Oncol* 2020;**13**:4

155. Fan H, Demirci U, Chen P. Emerging organoid models: leaping forward in cancer research. *J Hematol Oncol* 2019;**12**:142
156. Yang H, Sun L, Liu M, Mao Y. Patient-derived organoids: a promising model for personalized cancer treatment. *Gastroenterol Rep (Oxf)* 2018;**6**:243–5
157. Nagle PW, Plukker JTM, Muijs CT, van Luijk P, Coppes RP. Patient-derived tumor organoids for prediction of cancer treatment response. *Semin Cancer Biol* 2018;**53**:258–64
158. Lannagan TRM, Lee YK, Wang T, Roper J, Bettington ML, Fennell L, Vrbanac L, Jonavicius L, Somashekar R, Gieniec K, Yang M, Ng JQ, Suzuki N, Ichinose M, Wright JA, Kobayashi H, Putoczki TL, Hayakawa Y, Leedham SJ, Abud HE, Yilmaz OH, Marker J, Klebe S, Wirapati P, Mukherjee S, Tejpar S, Leggett BA, Whitehall VLJ, Worthley DL, Woods SL. Genetic editing of colonic organoids provides a molecularly distinct and orthotopic preclinical model of serrated carcinogenesis. *Gut* 2019;**68**:684–92
159. Stewart E, Federico SM, Chen X, Shelat AA, Bradley C, Gordon B, Karlstrom A, Twarog NR, Clay MR, Bahrami A, Freeman BB 3rd, Xu B, Zhou X, Wu J, Honnell V, Ocarz M, Blankenship K, Dapper J, Mardis ER, Wilson RK, Downing J, Zhang J, Easton J, Pappo A, Dyer MA. Orthotopic patient-derived xenografts of paediatric solid tumours. *Nature* 2017;**549**:96–100
160. Fusco P, Parisatto B, Rampazzo E, Persano L, Frasson C, Di Meglio A, Leslz A, Santoro L, Cafferata B, Zin A, Cimetta E, Basso G, Esposito MR, Tonini GP. Patient-derived organoids (PDOs) as a novel in vitro model for neuroblastoma tumours. *BMC Cancer* 2019;**19**:970