From The Department of Microbiology, Tumor and Cell Biology
Karolinska Institutet, Stockholm, Sweden

DEVELOPING NEW MODELS OF CHILDHOOD MALIGNANCIES USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Ana Marin Navarro

Karolinska Institutet

Stockholm 2019

The cover shows a three dimensional representation of iPS cells colonies stained for OCT4. This image was renderized by the great architects in GRAPH Visual Studio ©. This cover was design by the fantastic KOFFEE Comunicació Studio ©. Thanks for this amazing collaboration!

Barcelona 2019

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB 2019
Cover: iPS cells rendered by GRAPH Visual Studio © and cover design by KOFFEE Comunicació Studio ©

ISBN 978-91-7831-388-4

Developing new models of childhood malignancies using human induced pluripotent stem cells

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Ana Marin Navarro

Public defence: Friday 8th of March 2018,
9:30 am, CMB hall,
Berzelius väg 21

Principal Supervisor:
Associate Professor Margareta Wilhelm, Ph.D.
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Co-supervisor(s):
Associate Professor Anna Falk, Ph.D.
Karolinska Institutet
Department of Neuroscience

Professor Per Kogner, Ph.D, M.D.
Karolinska Institutet
Department of Women's and Children's Health

Opponent:
Davide Danovi, Ph.D, M.D.
King's College London
Department of Centre for Stem Cells & Regenerative Medicine

Examination Board:
Assoc. Prof. Lene Uhrbom, Ph.D.
Uppsala University
Department of Department of Immunology, Genetics and Pathology

Assoc. Prof. Lisa Westerberg, Ph.D.
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Docent Ras Trokovic, Ph.D.
University of Helsinki
Department of Biomedicum Stem Cell Center (BSCC)

To my beloved family; my mother Asun, my father Vicente, my brother Albert and my sister-in-law, Roser.

Also, to my old friend Anna McCarthy who I always remember and whom passed away from cancer.

To my friends, you know who you are!

A mi querida familia; a mi madre Asun, mi padre Vicente, mi hermano Albert y mi casi cuñada Roser.

También a mi vieja amiga Anna McCarthy, a quien siempre recuerdo y quien falleció de cáncer-

¡A mis amigos, que ya sabéis quien sois!

"*There is only one way to avoid criticism: do nothing, say nothing, and be nothing*."- Aristoteles

ABSTRACT

Early onset diseases such as childhood malignancies and neurodevelopmental disorders have been intricate to study. For many years, research has relied and dependent upon different animal systems. Despite the usefulness of these systems, which have allowed the understanding of the biology behind these processes, the differences between species are still an undoubted fact. The emergence of induced pluripotent stem (iPS) cell technology has indeed opened new venues for many fields including disease modeling, personalized cell therapy, and drug screening. iPS cells have the potential to virtually differentiate into any cell type, hence becoming an unlimited source of disease-relevant cell types. Here, we present examples demonstrating the potential of disease modeling using patient-derived iPS cells. Neuroblastoma (NB) and Medulloblastoma (MB) are both cancers linked to dysregulations in pathways important during human development. Whereas NB develops during the peripheral nervous system (PNS) development, MB initiates during central nervous system (CNS) development. We have taken advantage of the early developmental signature of iPS cells to model cancer. We used non-cancerous cells from patients carrying germline mutations in cancer predisposing genes, ALK and PTCH1, and developed *in vivo* models that offer a unique understanding of cancer initiation and progression. NB patients carrying an ALK germline mutation were used to generate iPS cells (Paper I) and subsequent differentiation to Neural Crest Cells (NCC) was performed (Paper II, III). For this, a NCC generation protocol was optimized using intermediate levels of BMP (Paper II). Next, labelled NCC from patients and controls were orthotopically transplantated into the adrenal gland of immunodeficient mice (Paper III). Mice were followed *in vivo* using IVIS system, and we detected increased luciferase signal after more than 8 weeks but no signal was observed in mice injected with control NCC. After a year, adrenal glands from mice were harvested and one case of ganglioneuroblastoma was diagnosed, suggesting a low penetrance and mild phenotype of ALK contribution in NB initiation. Using a similar workflow, we generated Neuroepithelial stem (NES) cells from iPS cells derived from Gorlin syndrome patients. Gorlin patients carry germline mutations in PTCH1. Mutations in PTCH1 constitutively activate the Sonic Hedgehog (SHH) signalling pathway. *In vivo* transplantation of patient cells into the cerebellum of immunocompromised mice showed faithful resemblance of human SHH MB. By establishing NES cell cultures derived from MB tumors in the mice cerebellum, we could show the potential use of this model for identifying new targets for cancer treatment (Paper IV).

Moreover, we exploited 2D and 3D human *in vitro* systems derived from iPS cells to study the role of p53 during early brain development. We show that p53 has an important function in maintaining the appropriate structure of human brain organoids. Moreover, we demonstrate that p53 maintains genomic instability and primes neural differentiation in human NES cells. Thus, revealing the role of p53 in a human *in vitro* context of brain development (Paper V).

In summary our work presents the big potential of iPS cell technology in the field of modeling disease.

POPULAR SCIENCE SUMMARY

Around the globe cancer is a devastating disease. Since many people think of cancer as an age-related disease, it is often forgotten that children can also suffer from it. Problems occurring during the early development of a child are most often the reason for the onset of cancer. Therefore, researchers try to understand what happens during that time. For ethical reasons, studying humans while they develop is restricted and limited. Therefore, researchers rely on studies that are performed in other species such as mice. Investigating diseases in species other than human comes with some disadvantages. For example it remains difficult to foresee possible side effects of a certain treatment or drug. Currently, the treatments that are used in children have a big impact leaving them with hormone problems and cognitive defects, implicating that new, better therapies are required. In 2012, a technique was developed that allowed researches studying human development in a dish. Because this technique was groundbreaking and offers new ways of conducting science it was awarded a Nobel Prize. From a small skin biopsy, fully mature cells could be reversed to an undifferentiated state by simply introducing four external factors. The resulting cells are named induced pluripotent stem (iPS) cells and they are equivalent to those that are present after fertilization. This means that these cells have now the potential to give rise to all the cells of the body. The technique was named cellular reprogramming. This opened a new era with a lot of potential for studying complicated diseases such as childhood malignancies that hopefully leave us with better treating options.

In paper I, II, III and IV of this thesis we reprogrammed mature cells, like skin cells, into an immature state in a dish. The obtained iPS cells have been derived from patients suffering either from Neuroblastoma (NB) or Medulloblastoma (MB), and carried mutations that are inherited through generations and that predispose to these cancers. NB and MB are two distinct cancers emerging in different locations of children. The first one is located close to the kidneys, in the adrenal gland, and sometimes spreads into ganglia and nerves of the peripheral nervous system, whereas MB is located in the cerebellum within the brain. iPS cells from these patients have been directed to a more mature state in the dish, hence creating a more differentiated state of cells – or progenitors - that are thought to be the source of the disease in each case. In order to understand how the cancer occurs, we injected these cells into the body compartment of the mice in which these tumors usually form. Therefore, progenitor cells from NB patients were injected into the adrenal gland and cells derived from MB patients into the cerebellum. After this, we observed and followed the injected cells to study if they could form tumors mimicking the human disease. This approach allows us to study more in depth what happens during cancer initiation. Moreover, it provides tools to investigate potential new targets that could be useful for future treatments.

iPS cells have been extensively used since their first appearance, and researchers have developed sophisticated tools to understand different diseases and general/their development. We used iPS cells from healthy individuals to derive three dimensional “mini brains” in the dish, to understand how a very important gene in tumor development has an effect during human brain development (**Paper V**).

LIST OF SCIENTIFIC PAPERS INCLUDED IN THE THESIS

I. **Ana Marin Navarro**, Kelly Day, Per Kogner, Margareta Wilhelm* and Anna Falk*.
Generation of induced pluripotent stem cell lines from two Neuroblastoma patients carrying a germline ALK R1275Q mutation
Stem Cell Research, 2018, 34,101356.

II. James Hackland*, Thomas J.R. Frith, Oliver Thompson, **Ana Marin Navarro**, Martin I.Garcia-Castro, Christian Unger and Peter Andrews.
Top-Down Inhibition of BMP Signaling Enables Robust Induction of hPSCs Into Neural Crest in Fully Defined, Xeno-free Conditions
Stem Cell Reports, 2017, 9, P1043-1052.

III. **Ana Marin Navarro**, Robin J Pronk, Daniel Bexell, Miklos Gulyas, Per Kogner, Anna Falk and Margareta Wilhelm.
A neuroblastoma model using patient-derived induced pluripotent stem cells carrying a R1275Q germline mutation.
Manuscript

IV. Evelyn Susanto, **Ana Marin Navarro**, Anders Sundström, Jignesh Tailor, Jonne Rietdijk, Jens-Martin Hübner, Irina Alafuzoff, Ann Nordgren,Thierry Magnaldo, Peter Siesjö, John Inge Johnsen, Marcel Kool, Anna Darabi, Fredrik Swartling, Anna Falk*, and Margareta Wilhelm*.
Modelling of medulloblastoma and identification of therapeutic targets with patient iPS cell-derived neural stem cells.
Manuscript in revision.

V. **Ana Marin Navarro**[§], Robin J Pronk[§], Astrid van der Geest, Ganna Oliynyk, Marie Arseninan-Henriksson, Anna Falk* and Margareta Wilhelm*.
p53 maintains cellular organization in human brain organoids and controls genomic stability and temporal differentiation of human neural stem cells.
Manuscript in submission

PUBLICATIONS NOT INCLUDED IN THE THESIS

I. **Ana Marin Navarro**, Evelyn Susanto, Anna Falk and Margareta Wilhelm*.
Modeling cancer using patient-derived induced pluripotent stem cell to understand development of childhood malignancies.
Cell Death Discovery, Review, 2018, 4(1): 7.

II. Elias Uhlin, **Ana Marin Navarro**, Harriet Rönnholm, Kelly Day, Malin Kele, Anna Falk*.
Integration free derivation of human indued pluripotent stem cells using laminin 521 matrix
J Vis Exp, 2017, (125).

III. Marina Stantic, Habib A. M. Sakil, Hanna Zirath, Trixy Fang, Gema Sanz, Alejandro Fernandez-Woodbridge**, Ana Marin Navarro,** Evelyn Susanto, Tak W. Mak, Marie Arsenian Henriksson, and Margareta T. Wilhelm*.
TAp73 suppresses tumor angiogenesis through repression of proangiogenic cytokines and HIF-1α activity
PNAS, 2015, 112 (1) 220-225.

* Corresponding
§ Equal contribution

CONTENTS

LIST OF ABBREVIATIONS

2D	Two-Dimensional
3D	Three-Dimensional
ALCL	Anaplastic Large Cell Non-Hodgkin's Lymphoma
ALK	Anaplastic Lymphoma Kinase
APC	Adenomatous Poliposus Coli Protein
BAX	BCL2 Associated X
BCL-2	B-Cell Leukemia/Lymphoma 2
bFGF	Basic Fibroblast Growth Factor
BMP	Bone Morphogenetic Protein
CCHS	Congenital central hypoventilation syndrome
CDK6	Cyclin Dependent Kinase 6
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CNS	Central nervous system
CTD	C-terminus Domain
CTNNB1	Catenin Beta 1
DBD	DNA Binding Domain
DNA	Deoxyribonucleic acid
EB	Embryoid Bodies
ECM	Extracellular Matrix
EGL	External granule layer
EMT	Epithelial-to-mesenchymal transition
ES	Embryonic Stem Cell
FBS	Fetal Bovine Serum
FGF	Fibroblast Growth Factor
GCP	Granule Cell Progenitors
GLI1	Glioma Associated Oncogene Homolog 1
GLI2	Glioma Associated Oncogene Homolog 2
i17q	Isochromosome 17 q
ICM	Inner Cell Mass
IDFRs	Image-Defined Risk Factors

INM	Interkinetic Nuclear Migration
INRG	International Neuroblastoma Risk Group
INRGSS	International Neuroblastoma Risk Group System
INSS	International Neuroblastoma Stage System
iPS	Induced Pluripotent stem cell
KLF4	Kruppel-like factor 4
KO	Knock Out
LCA	Large-Cell Anaplastic
LFS	Li-Fraumeni Syndrome
LN-521	Laminin 521
MB	Medulloblastoma
MBEN	Medulloblastoma with extensive nodularity
MDM2	Mouse Double Minute 2
MEFs	Mouse Embryonic Fibroblasts
MEFs	Mouse Embryonic Feeders
mRNA	Messenger Ribonucleic Acid
NANOG	Nanog Homeobox
NB	Neuroblastoma
NBCCS	Nevoid basal cell carcinomas syndrome
NCC	Neural Crest Cells
NES	Neuroepithelial Stem Cells
NSC	Neural Stem Cells
OCT3/4	Octamer-binding Transcription Factor 4
OD	Oligomerization Domain
OSKM	Oct4, Sox2, Klf4, c-Myc
OTX2	Orthodenticle Homeobox2
PDACs	Pancreatic Ductal Adenocarcinoma
PHOX2B	Paired like homeobox 2B
PNS	Peripheral Nervous System
PTCH1	Protein Patched Homolog 1
PUMA	p53 upregulated modulator of apoptosis

RB	Retinoblastoma
RG	Radial Glia
RNA	Ribonucleic Acid
RTK	Receptor Tyrosine Kinase
SCs	Stem Cells
SFEB	Serum-free Culture of Embryoid body-like aggregates
SHH	Sonic Hedgehog
SMO	Smoothened
SOX2	Sex determining region Y-box2
SSEA-4	Stage-Specific Embryonic Antigen 4
SUFU	Suppressor of fused homolog
SVZ	Subventricular Zone
SeV	Sendai Virus
TAD	Transactivation Domain
TERT	Telomerase Reverse Transcriptase
TGF-β	Transforming Growth Factor Beta-1
VZ	Ventricular zone
WNT	Wingless

1 INTRODUCTION

1.1 CANCER

The terminology of Cancer originates from ancient times, back to olden Greece more than thousands of years ago. Hippocrates (460 – 370 BC), a Greek physician, has often been referred to as the father of medicine. He named tumors or ulcers, ***carcinos***, the Greek word for crab. It is thought that the name was probably adopted because of his empirical visualizations of tumor shapes and their adhesion to surrounding tissues within the body. Later on, carcinos was translated to Latin therefore, we refer to it as Cancer today.

Cancer is an incredibly complex and heterogeneous disease that emerges and develops from normal tissues and is undoubtedly one of the leading causes of death worldwide [1]. By the end of 2018, 9.6 million cancer related deaths were estimated (https://www.who.int/cancer/). Cancer incidence is increasing globally with obvious potential implications in the health care system. Tumors can arise from many different specialized cells that originate from all three germ layers of the vertebrate embryo indicating the huge variety and spectrum of this disease.

1.1.1 HALLMARKS OF CANCER

The paths that a normal cell can undertake to become malignant are multiple and intricate. Normal cells are tightly regulated, and especially their proliferation is securely controlled. For the past centuries, researchers put a lot of effort into understanding the mechanisms governing tumorigenesis, to find new ways to target them and improve patient survival. Postulating that cancer is a multi-step disease and that normal cells undergo several transformation events to become malignant, Hanahan and Weinberg defined six different biological characteristics - or hallmarks - that can initiate cancer development in normal tissues [2]. Cancer cells have the potential to sustain proliferative signaling (1), evade growth suppressors (2), resist cell death (3), enable replicative immortality (4), induce angiogenesis (5), and activate programs of invasion and metastasis (6). All these acquired features can allow the cancer cell to progress. Indeed, these characteristics can be governed by different mechanisms and at a different time during cancer progression. As knowledge of the cancer field increased, new enabling hallmarks and emerging characteristics were added to this picture. Ten years later, four other categories were added to the already established hallmarks of cancer; Genomic instability allows the cell to harbor mutations that could potentially lead to an advantage over normal cells. A second characteristic involves the reaction of the immune system to promote tumorigenesis. And last but not least, two other emerging hallmarks were described; the ability of cancer cells to reprogram energy metabolism and to be able to evade the immune system (Figure 1) [2].

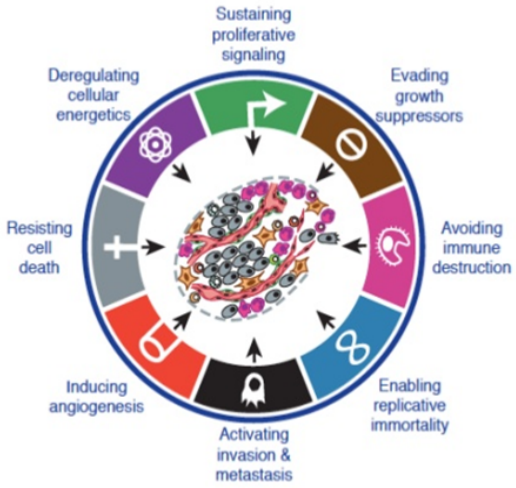

Figure 1. Hallmarks of cancer-Next generation. Modified from Hanahan and Weinberg 2011, Cell, with permission from the publisher.

1.1.2 CHILDHOOD CANCER VS ADULT CANCER

Childhood cancers are rare compared to adult cancers, however in developing countries cancer is still the world leading cause of death in children and adolescents between 0 and 19 years. The most common childhood cancers include leukemias, brain tumors, lymphomas, neuroblastoma and sarcomas [3] (Figure 2).

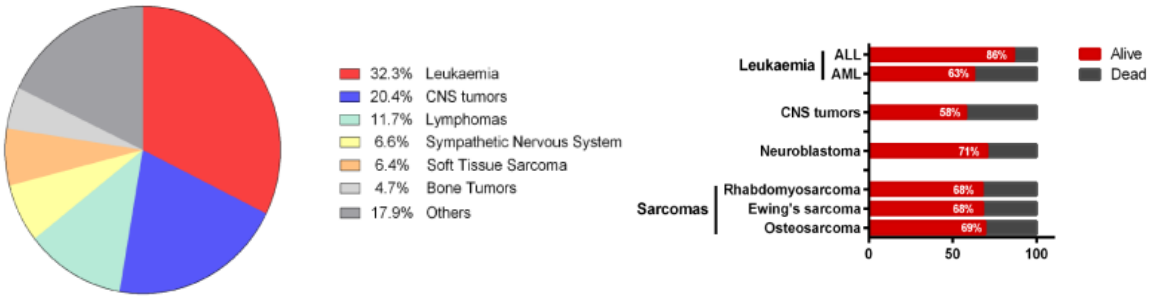

Figure 2. Incidence and mortality of childhood cancers. Incidence is shown in the left panel and mortality in the right panel. Reproduced from Marin Navarro et al., 2018, Cell Death Discovery with permission from the publisher.

However, age and incidence are not the only difference between childhood malignancies and adult cancers. The cell of origin, genetic and epigenetic abnormalities and the tumor microenvironment usually differ between children and adult tumors [4,5]. For example, whereas adults mainly suffer from carcinomas, which have an epithelial origin, children's cancer are mostly hematological or central nervous system (CNS) tumors. These malignancies are considered to have an embryonic origin and probably arise in stem or progenitor cells during development. Therefore, childhood cancers can be described as developmental disorders. The early onset of appearing abnormalities could be an explanation why childhood cancers develop faster and harbor fewer mutations than adult cancers [6]. Despite the fact that the 5-year cancer survival rate of childhood cancer in general is about 80%, which is significantly better than adults, some childhood cancers such as CNS tumors, have a low survival rate (Figure 2). The most common treatments for childhood malignancies are chemotherapy, surgery and radiation. During the last decade the improvement of treatments for childhood

cancers has been extensive. However, despite improved survival rates, treatments can still lead to permanent side effects including hormone dysregulation, growth and cognitive effects [7, 8]. For this reason, focusing on finding new treatments to target childhood malignancies becomes paramount.

1.1.3 NEUROBLASTOMA

Neuroblastoma (NB) is a rare embryonic cancer but the most common extracranial solid tumor during childhood. NB accounts for around 7 % of all childhood cancers (Figure 2). In Sweden, every year approximately 15 to 20 cases are diagnosed whereas in the United States around 650 cases appear (Childhood Cancer incidents and survival in Sweden 1984-2010). NB develops in the peripheral nervous system (PNS) and primary tumors are usually found in the abdomen, where they are commonly observed in the medulla of the adrenal glands but also in the sympathetic ganglia, thus they can also be detected in the neck, chest and pelvic regions [9].

1.1.3.1 STAGING AND CLASSIFICATION

NB can be classified according to their predicative behavior which relates to genetic changes of the tumor. The most widely and currently used system is described in the International Neuroblastoma Staging System (INSS) [10, 11]. INSS is a post-surgery system and the diagnose is determined just after removal of the tumors. Therefore, the INSS classification has limitations for children who do not need or cannot have surgery. The system classifies NB in different stages; 1, 2 (A, B), 3, 4 and 4S. Stage 1 describes that the tumors are localized and completely removed without presence of metastasis. Subsequent stages worsen up to stage 4 which defines more aggressive tumors with presence of metastasis. Stage 4S has special characteristics that include children younger than one year with spread of cancer to other organs like liver, skin and bone marrow containing less than 10% of tumor cells. Frequently tumors of the 4S category regress spontaneously, a special attribute not seen in many cancers. However, due to the limitations of the INSS an alternative staging system was presented by the international NB risk group (INRG) in 2009. The INRG staging system (INRGSS)[12], aimed to help researchers to compare their studies across the world. The INRGSS is based on image-defined risk factors (IDFRs) present on the imaging tests that allow to distinguish the difficulty of tumor removal. The stages are L1, L2, M and MS. In short, category L1 depicts that the tumor is well localized and contained and absent of IDFRs. Presence of IDFRs indicates L2 stage. The metastatic tumor grade is categorized within the M stage. If the tumor has metastasized strictly to skin, bone marrow or liver in children younger than 18 months it belongs to the MS stage, similar to the 4S stage in INSS. Together with other prognostic factors such as age, histology, DNA ploidy (DNA content of cancer cell), MYCN gene amplifications, chromosomal changes (usually deletion of 1p and 11q) and neurotrophin receptors status, the INRGSS provides a complete classification that separates patients into very low, low, intermediate and high risk groups. Based on this classification the nature of the cancer is identified and the best treatment strategies selected [12]. Histology of NB is a prognostic tool used for NB classification. Usually divided in stromal component; Schwannian stroma rich, dominant or poor. Schwannian stroma-poor tumors, which are the most aggressive ones, are sub-divided in morphologic features; undifferentiated, poorly

differentiated and differentiating subtypes. Calcification, and mitotic rates are also taken in consideration in stroma-poor tumors [13].

1.1.3.2 THE ORIGIN OF NEUROBLASTOMA

Elucidating the origin of NB is crucial to understand tumorigenesis. Because of its early onset and location of the tumors, NB it is thought to originate from PNS precursor cells [14]. However, for obvious reasons studying the initiation of NB is difficult in humans. The majority of NB tumors arise anywhere along the sympathetic chain but most frequently in the adrenal medulla and paraspinal ganglia [15]. The cells who contribute to the sympathetic nervous system during development are the neural crest cells, therefore thought to be involved in the origin of NB [16].

1.1.3.3 THE NEURAL CREST CELLS

The neural crest cells (NCC) were first discovered by the embryologist Wilhelm His in 1868. He described a cell population that emerges between the dorsal ectoderm and the neural tube border and is uniquely present during vertebrate embryogenesis [17]. This incredible multipotent and transient cell population resides in the elevating neural folds in the dorsal neural tube until its closure during development (Figure 11 and 12 / Section 1.2.4.2). Subsequently, they undergo an epithelial-to-mesenchymal transition (EMT), migrating out from the neuroepithelium to different distant places along the embryo. Their multipotent capacity is defined by their potential of giving rise to a wide range of tissue types including craniofacial skeleton, pigmented cells, neurons, as well as glia among other cell types. NCC can originate from different parts of the embryo neuroaxis, which is segmented and gives rise to different NCC derivatives; cranial, cardiac, vagal, and trunk. Cranial NCC are a good example of the multipotency of NCC. They give rise to the majority of the mesenchymal derivatives such as bone and cartilage of the head and face but also pigmented cells, smooth muscle cells, nerves, glia, and connective tissue. Cardiac NCC contribute to heart development by forming the aorticopulmonary septum of the heart. Vagal NCC are responsible for the enteric nervous system of the gut and finally, the trunk NCC will differentiate into neurons and glia of the PNS, secretory cells, and pigmented cells [18].

Most of the studies conducted to understand NCC development and migration have been carried out in chicken embryos. NCC develop upon different signaling pathways in the dorsal/ventral axis of the embryo including BMP and SHH signaling pathways present in the developing embryo [19]. These studies demonstrated that during migration of the avian neural crest cells, signals from the dorsal aorta, such as BMP could determine the fate of NCC into the specific sympathoadrenal linages including the chromaffin cells or sympathetic neurons [20, 21, 22, 23].

1.1.3.4 GENETIC PREDISPOSITION TO NEUROBLASTOMA

Familial NB is rare and accounts for less than 2% of all NB [24]. Some mutations related to signaling pathways that are known to be important during development are linked to familial developmental syndromes and NB [25, 26]. For example, PHOX2B is a homeodomain-containing protein which is expressed throughout the developing sympathetic, parasympathetic and

enteric ganglia [27]. The importance of PHOX2B is underlined by loss of function mutations that lead to congenital central hypoventilation syndrome (CCHS) and/or Hirschsprung disease. These are neurocristopathies that are also found in NB patients. Moreover, PHOX2B was identified as a predisposition risk factor for NB [28, 29], however, mutations in this gene have only been found in a small subset of hereditary NB and mostly associated with neural crest derived disorders. Besides PHOX2B, other familial mutations have been found of which ALK mutations are more common. Around 75% of all NB familial cancers present a gain-of-function of ALK which makes ALK the main predisposing gene for familial NB [30].

1.1.3.5 ALK, DEVELOPMENT AND NEUROBLASTOMA

Receptor Tyrosine Kinases (RTK) are a large family of surface receptors that control multiple cellular processes which are active during development [31] and also tumorigenesis [32]. Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor [33] (Figure 3) that is expressed throughout the developing nervous system [34]. However, the physiological role in human is still not entirely understood although ALK fusion proteins, overexpression, and activating mutations are involved in carcinogenesis of different types [35]. Addressing the incomplete understanding of ALK, studies in ALK knock out (KO) mice, have described effects in neurogenesis and in hormonal regulation [36, 37]. ALK was first discovered as a chromosomal translocation occurring between 2;5 in anaplastic large cell non-Hodgkin's lymphoma (ALCL). This translocation results in a fusion protein which is constitutively active [38]. Mainly, downstream ALK signaling pathways have been characterized mostly from fusion proteins occurring in cancer. In general, ALK as a RTK is responsible for activating different signaling pathways such as PI3K-AKT, JAK-STAT and MAPK pathways that lead to cell growth, survival or differentiation depending on the cellular context [35, 39].

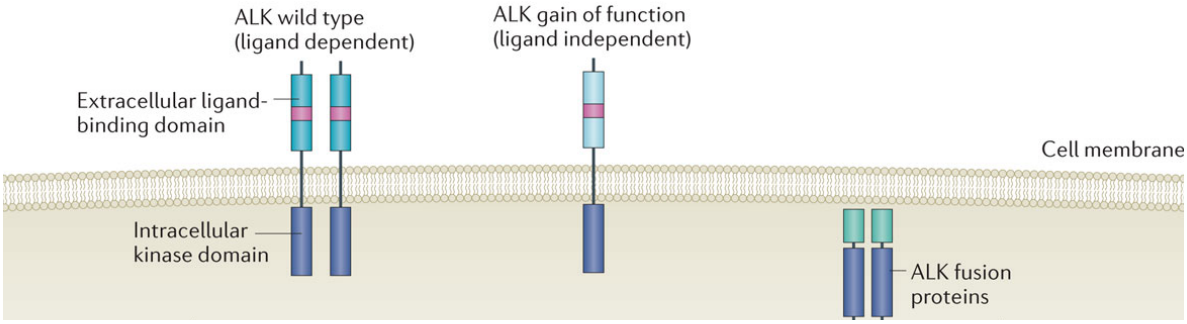

Figure 3. ALK structure and domains. From Hallberg and Palmer, 2013, with permission from the publisher. Cancer Nature Reviews.

1.1.3.6 ALK MUTATIONS

Gain-of-function mutations in ALK have been found in sporadic [40, 41] and familial NB [42, 43] but also in inflammatory myofibroblastic tumors and non-small-cell lung cancer (NSCLC). Mutations in the kinase domain of ALK occur in around 8% of all NB (Figure 4) [44]. Two hot spot mutations are frequently observed in NB; F1174 and R1275, the latter being the most frequent (85%) in NB patients. ALK R1275 mutation, is a single-nucleotide substitution within the activation loop of the kinase domain that leads to changes in the amino acid residues (Figure 4). The substitution of arginine to glutamine or leucine results in autophosphorylation

and autoactivation of the receptor [41]. Whereas R1275 mutations can be introduced both in germline and somatically [42], F1174 mutations has solely been found in sporadic tumors [43]. Importantly, cohort studies showed that ALK mutations are associated with poorer survival in intermediate and high risk cases [44]. Moreover, genome sequencing analysis revealed that relapsed NB cases have an increased frequency of ALK mutations [45] . Of note, F1174 mutations display a higher tumorigenic potential than other ALK mutations [46, 47] but both F1174 or R1275 mutations contribute to and cooperate with MYCN during tumor formation [47, 48].

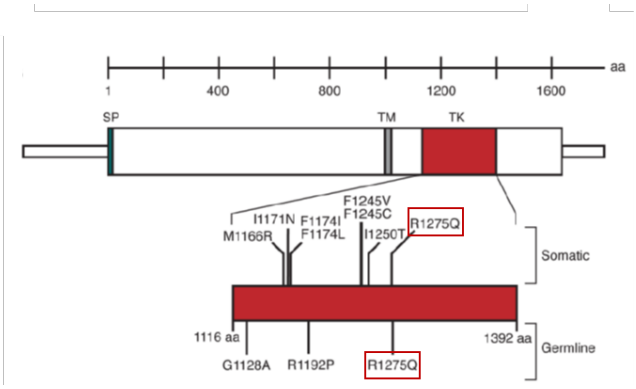

Figure 4. ALK somatic and germline mutations. Modified from Mossé et al, 2008, Nature, with permission of the publisher.

1.1.3.7 PRECLINICAL MODELS

Multiple NB models have been developed and established in order to gain more understanding of NB initiation and progression. Preclinical models are essential to develop new therapeutic strategies useful in clinical trials. For example, animal models including transgenic mouse models and xenografts have been fundamental for basic research in solid pediatric tumors [49].

MYCN was identified more than 40 years ago and MYCN amplifications were found to correlate with poor prognosis in NB patients. Today, MYCN amplifications are still used to identify high risk cases [50]. Therefore, a lot of focus has gone into understanding MYCN contribution in NB development. The most widely used tools are the TH-MYCN transgenic mouse model where MYCN is driven by a rat tyrosine hydroxylase gene (TH) [51], and the MYCN Cre-conditional mouse model that conditionally expresses MYCN in dopamine beta hydroxylase positive cells, like NCC [52]. However, despite they have histological and pathological resemblance to human NB, the mouse models have limitations [53]. For example, NB penetrance varies depending on the background of the mouse strain, as well as frequency of bone marrow metastasis which is often seen in human NB but low in mice. As an alternative, xenografts into immunocompromised mice using human NB cell lines can be used. One disadvantage of this model is the location of the injected cells (ordinarily subcutaneous injections) which could potentially affect tumor-host interactions. Therefore, orthotropic xenografts, which are injected into the tumor specific site of origin, have been used. However, NB cell lines used for xenograft are often cultured *in vitro* for many years leading to inclusion of more unknown variables. To overcome this, other methods such as patient derived xenografts (PDX) models have been established [54]. In this case, human tumor material is directly transplanted into mice. PDX models are thought to be more predictive for clinical relevance than cell line based xenografts.

1.1.4 MEDULLOBLASTOMA

Brain tumors and other CNS malignancies are the most common cause of death in children (0-15 years old) [55] (Figure 2). Medulloblastoma (MB) is a malignant embryonic tumor arising in the cerebellum and one of the most frequent cancers among children [56]. The prognosis of MB has improved during the past decades. Resection of the tumor, chemotherapy, and whole brain and spinal cord radiation are first-front used therapies [57]. However, patients suffering from relapse need new therapeutical approaches. Moreover, current treatments might leave MB survivors with permanent deficits as neurocognitive defects and physical disabilities [58].

1.1.4.1 CLASSIFICATION OF MEDULLOBLASTOMA

MB can be histologically and molecularly classified. Histologically MB is divided in the classic, nodular desmoplastic, large-cell anaplastic (LCA), and MB with extensive nodularity (MBEN) subtypes [59]. The classic histology subtype is characterized by poorly differentiated, small and round-tumor cells whereas nodular desmoplastic tumors show nodular areas, known as pale islands, due to their white appearance. Nodular regions are usually surrounded by connective tissue. Additionally, the presence of reticulin rich components and neuronal markers can be observed. Patients with nodular desmoplastic tumors, that are often found in infants (less than 4 years old) and adults (more than 16 years old), generally have a better prognosis than patients with a classic subtype. The LCA subtype displays cells with enlarged nuclei, prominent nucleoli, and different amounts of cytoplasm. Moreover, tumors are characterized by presence of proliferation and apoptosis. LCA results in the poorest outcome and is classified as high risk MB. MBEN shows nodular regions with no presence of reticulin between the nodules. It is mostly prominent in infants and has a better prognosis than the rest of subtypes [60, 61 62].

The era of transcriptomics has helped to develop new research tools to study tumor heterogeneity in more in depth. In addition to histological MB subtypes, gene signature profiling identified 4 additional subgroups of MB: Wingless (WNT), Sonic Hedgehog (SHH), Group 3 and Group 4 (Figure 5) [63 64]. WNT and SHH groups are named due to the respective dysregulation in major signaling pathways and only exhibit minimal overlap based on methylation profile studies and transcriptomic analysis [65]. Group 3 and 4 are more similar to each other and therefore an additional substructure its needed to clarify their differences. Recently, this classification has been revised and different sub-categories have been proposed based on distinct somatic copy-number aberrations, differentially activated pathways and variable clinical outcomes (Figure 5) [66].

WNT Subgroup: WNT MB consists of tumors with abnormalities in Wnt/B-catenin signaling [67]. Almost 90% of the tumors in this subgroup present somatic mutations of the CTNNB1 gene which encodes the β-catenin protein. Mutations of CTNNB result in a constitutive activation of the WNT pathway leading to increased proliferation [68]. In addition, the WNT subgroup harbors germline mutations in APC, a Wnt inhibitor [69] that is often associated with Turcot Syndrome. However, the 5-year survival rate of patients within the WNT subgroup is surprisingly good (Figure 5) [70 66]. A recent study conducted analysis of gene expression and

methylation signatures of WNT MB patients. Cavalli and colleagues identified two different subcategories within the WNT type. The WNTα subcategory that is comprised of children with monosomy in chromosome 6 and WNTβ, that involves older patients with diploidy in chromosome 6 (Figure 5) [66].

SHH Subgroup: Similarly to WNT group, SHH MB are named after the activation of SHH signaling pathway in the etiology of this group. The incidence in SHH group is higher in both infants and adults with intermediate risk. The histological subtype is usually limited to nodular/desmoplastic subtypes which makes it easier to identify tumors in the SHH subgroup [64]. The SHH subgroup contains germline mutations associated to different syndromes such as Nevoid basal cell carcinomas syndrome (NBCCS) and Li-fraumeni syndrome. NBCCS, also named Gorlin syndrome, patients can harbor PTCH1 mutations (Figure 6), therefore mutations affecting the SHH receptor which are often be associated with NBCCS and MB. PTCH1 mutations are found in 10% of all MB [71]. Equally, germline mutations in SUFU have been linked to MB and NBCCS and are sporadically found in MB [72 73]. Li-fraumeni syndrome patients who carry p53 germline mutations are predisposed to develop MB. Other SHH signaling pathway components including Smoothened (SMO), GLI1 and GLI2 have been found sporadically mutated in MB [64]. The most recent classification distinguishes four SHH subgroups based on a cohort of 223 SHH MB patients; SHHα tumors affect children and have the worst prognosis. The SHHα subgroup is enriched with MYCN and GLI2 amplifications and characterized by TP53 mutations all of which indicate a high risk of MB. Infant SHH MB is classified in two subtypes; SHHβ and SHHγ. The SHHβ subtype is generally considered to have a higher metastatic potential than the SHHγ subtype. SHHβ harbors more PTEN deletions whereas the SHHγ subtype does not show metastases, less genomic instability, and fewer amplifications. Subtype SHHδ includes adults and has a better prognosis. It is mainly defined by enriched TERT promoter mutations [66].

Group 3: Group 3 MB have the worst prognosis of all subgroups. It is characterized by classic and LCA histology and often increased metastases. MYC overexpression and amplifications account for almost 20% of the tumors graded as group 3 MB. Additionally, OTX2 amplifications [63], chromosomal rearrangements that include isochromosome 17q (i17q), gain of 1q and loss of chromosomes 11 and 10q are typical alterations in group 3 MB [74]. Cavalli et al., identified 3 different subtypes of group 3 MB; subtypes 3α and 3β have a better prognosis compared to 3γ and exhibit a similar metastatic frequency. Subtype 3γ has the worst prognosis independently of MYC amplifications, therefore expanding high-risk MB beyond the MYC status of a patient (Figure 5) [12].

Group 4: Group 4 is the most prevalent type accounting for 40% of all MBs. As well as group 3 the tumors are characterized by a classic histology and rearrangements of isochromosome 17q. Similarly, three different subtypes within group 4 have been identified; Subtype 4α often shows MYC and CDK6 amplifications. Duplications in SNCAIP are representative of subtype 4β whereas subtype 4γ also includes CDK6 amplifications [66].

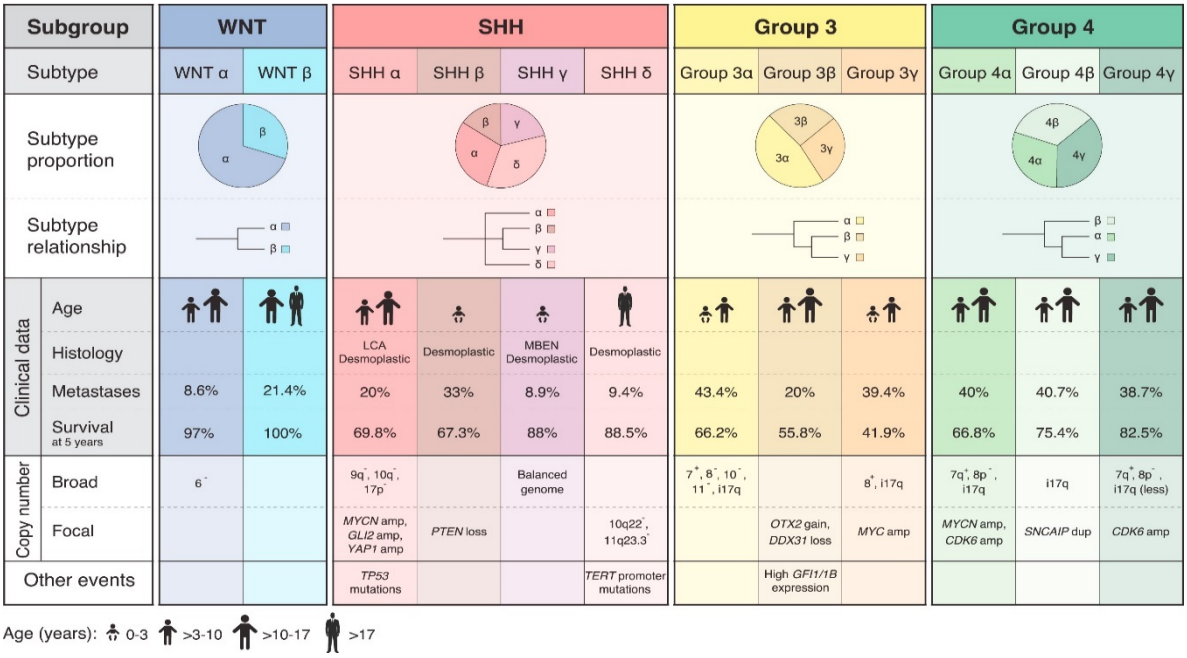

Figure 5. Molecular subtypes of MB. Reprinted from Cavalli et al, Cancer Cell, 2017, with permission from publisher.

1.1.4.2 CEREBERAL DEVELOPMENT, SHH and MB

As many other developmental diseases in humans, identifying the cell of origin of MB has been challenging and is still a matter of debate. However, there are evidences that the origin of MB is related to abnormalities during cerebral development. The development of the cerebellum is an intrinsic process beginning in early embryonic stages [75]. As we know today, the CNS originates from the emerging epithelium of the neural tube early during development. During early development, the neural tube transforms drastically generating the three brain regions; the forebrain (Prosencephalon), the midbrain (Mesencephalon) and the hindbrain (Rhombencephalon). Hindbrain specification will give rise to the rhombic lip that plays an important role in the cerebellar neural system [76]. The cerebellum consists of a cortical structure, subcortical nuclei and a cerebellar nucleus which projects neurons to their targets [77]. The cerebellar cortex has a simple laminar structure which contains different types of neurons, the granule cells, the Purkinje cells, and a diverse range of interneurons which coordinate the correct connectivity of the cerebellum. Cerebellar neurons are generated from two regions; the external granule layer (EGL) and the ventricular zone (VZ). Research in the past decades has shown that granule cells are produced by granule cell progenitors (GCP) occurring in the EGL which was initially formed from the rhombic lip.

The SHH signaling pathway is known to control proliferation of the EGL progenitors [78]. Secreted SHH binds to PTCH, which is highly expressed in EGL precursor cells, thereby ceasing PTCH inhibition of SMO (Figure 6). Subsequently, SMO activation leads to the release of GLI transcription factors from the cytosolic repressor SUFU and translocation of GLI into the nucleus where it drives target gene expression. GLI transcription factors drive the expression of genes that are important for proliferation - like MYC, MYCN and CyclinD1 - , angiogenesis, apoptosis, EMT, self-renewal, and for the regulation of the pathway such as

GLI1 and PTCH [79]. The SHH subgroup of MB is well defined because of constitutive activation of SHH pathway due to mutations in genes regulating GLI activation (See section 1.1.4.1). Gorlin syndrome patients harbor germline mutations in PTCH1 or SUFU and are predisposed to develop MB showing the importance of the SHH signaling pathway in the development of MB.

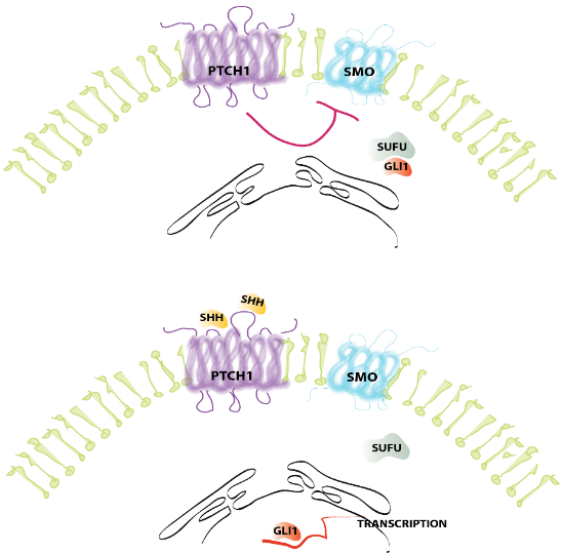

Figure 6. Schematic of SHH signaling pathway. (Top) Inactive pathway occurs without the presence of SHH ligand. Therefore, PTCH1 inhibits SMO resulting in GL1 being sequestered by SUFU. (Bottom) SHH binds to PTCH1 realizing SMO that leads to accumulation of GLI1. GLI1 activates several target genes linked to proliferation, self-renewal and suppression of apoptosis.

1.1.4.3 MODELS OF MB

Pre-clinical MB models have been of importance for understanding mechanisms behind MB. Advantages of established MB cell lines are their easy usage and possible continued culture. However, this can have potential implications due to selection bias or genetic drift of the cell lines [80]. Recently, an attempt to classify the current established MB cell lines into MB subgroups has been made. Of 44 available cell lines, less than half have been classified. The most common classified cell lines are MYC amplification bearing cell lines of group 3 MB. Currently, there are four SHH-MB established cell lines DAOY [81] UW228, UW426 and ONS-76 [82]. Half of them harbor p53 mutations (DAOY and UW-228) [83]. Alternatively, tumor patient-derived tumor cells can be cultured and used in mice for xenografts experiments. Moreover, mouse models have been established with the purpose of understanding MB progression and the vast majority mimic the SHH subgroup [64, 84]. Mice engineered with Ptch1 mutations, knocking out Gli1, or overexpressing SMO all demonstrated a contribution to the development of SHH MB [85, 86, 87]. Equally, expression of MYC in combination with p53 loss led to aggressive or high risk MB [88]. Mice bearing CTNNB1 mutations mimicked WNT MB. However, metastasis usually is the main cause of death in children suffering from MB, which most of mouse models do not faithfully recapitulate, suggesting other models are needed.

1.2 WHY USE INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELING?

This section will discuss basic stem cell biology concepts in order to understand the use of induced pluripotent stem (iPS) cells for disease modeling.

1.2.1 STEM CELLS

Embryogenesis

Human development begins when an oocyte gets fertilized by a sperm cell and fusion of genetic materials occurs. The resulting cell, called the zygote, is capable of generating an entire organism by rapid division of the zygote into identical cells, a process called cleavage. The derived structure of 16 identical cells is called Morula. After compactation and differentiation of the cells, the blastocyst is created. The inner cell mass (ICM) forms within the blastocyst whereas the trophoblasts are the cells forming the outer layer. The blastocyst establishes prior to uterus implantation. The ICM is the only pluripotent lineage in the cavity and develops the embryonic and extra-embryonic tissues of the organism. The trophoblast is responsible to provide nutrients to the embryo giving rise to the placenta (Figure 7). During the second week, the ICM will form the epiblast and the hypoblast, forming two different cavities; the primitive yolk sac and the amniotic cavity [89]. Gastrulation (week 3) is the process in which the gastrula is rearranged and results in the formation of the three germ layers. The three germ layers form all the tissues and organs of the body. The three germ layers are, the ectoderm, the mesoderm and the endoderm. (1) The **ectoderm** originates the epidermis, the CNS, and the peripheral nervous system such as ganglia and nerves. (2) The **mesoderm** forms the dermis of the skin, muscle, connective tissue, bone, kidneys, ureters, the lymph, and the spleen. (3) The **endoderm** generates organs such as stomach, intestine, colon, liver, pancreas, bladder, trachea, esophagus, and the lungs. In section 1.2.4.1 I will focus briefly on the formation of the neural plate from the ectoderm, which creates the CNS. (4) Neural crest cells (NCC) are sometimes referred to as the "**fourth germ layer**". NCC emerge from the dorsal part of the neural tube, however, the non-neural ectoderm and mesoderm are also required for their formation [90]. NCC will be as well discussed in more detail.

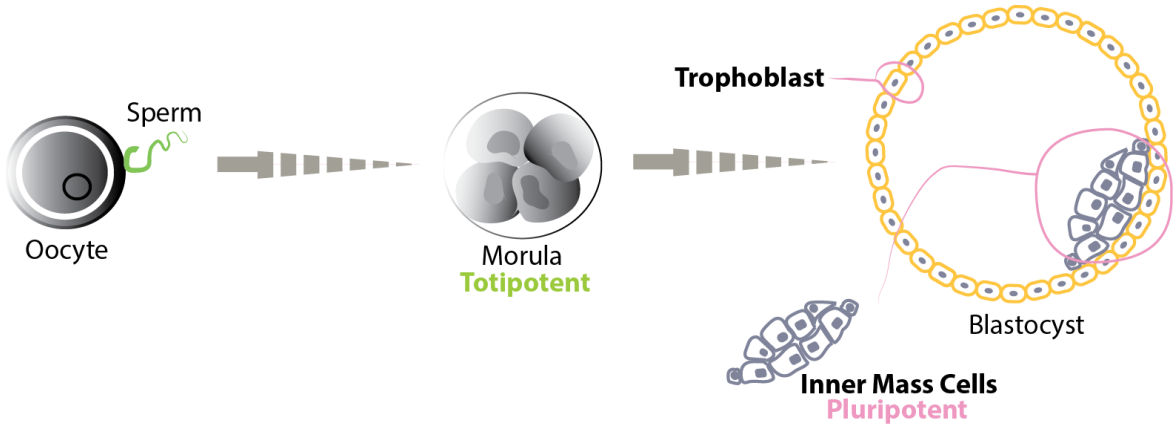

Figure 7. Scheme of Blastocyst formation from a Zygote. To the left, the oocyte is fertilized by a sperm cell. Division of the zygote into identical cells occurs, Morula (middle). To the right, the blastocyst, in yellow the trophoblast and in gray the ICM are shown.

What is a stem cell?

A cell with the capability of replicating itself (Self-renewal) and the ability to give rise to more specialized cell types (Differentiation) is defined as a stem cell (SC). SCs are found in multi-cellular organisms where they exhibit their characteristics, during development or being resident within various tissues providing a new source for more differentiated cells. SCs are classified based on their differentiation potential. (1) **Totipotent**: SCs that can generate all three germ layers and the extra-embryonic tissue called the trophectoderm which gives rise to the placenta. During development the zygote, after fertilization, is considered totipotent (Figure 7). (2) **Pluripotent**: SCs from the ICM of a pre-implantation blastocyst (Embryonic stem cells) or reprogrammed by defined factors, as described in section 1.2.3 (iPS cells) (Figure 7). These SCs are able to generate the three germ layers and germ cells but not extra-embryonic tissue. (3) **Multipotent**: Multipotent SCs demonstrate even more restricted potential and are only able to differentiate into specialized cell types. For example, organs that are exposed to constant turnover like the hair follicle, skin epidermis, and the intestinal crypt contain multipotent tissue resident SCs maintaining the respective organ.

1.2.2 EMBRYONIC STEM CELLS AND IPS CELLS

Embryonic Stem Cells

Embryonic stem (ES) cells are pluripotent stem cells derived from the ICM of the embryo (Figure 7). About 30 years ago, in 1981, the first ES cells were isolated from a mouse blastocyst [91] and they could be cultured on mouse embryonic fibroblasts (MEFs) [92, 93]. Almost 10 years later, it was possible to isolate ICM cells from the human blastocyst [94] and passage them long term in cell culture systems [95]. The maintenance of cells that are able to differentiate into a distinct range of specialized cell types *in vitro* bears huge therapeutic possibilities [96]. However, for that to be possible, a lot of focus has been into developing better cell culture conditions. Hallmarks of successful ES cell culture are; (i) indefinite self-renewal, (ii) maintaining a normal euploid karyotype over extended cultures, (iii) expression of pluripotency markers (iiii) and to sustain differentiation potential into derivatives of all three germ layers [96, 97]. Different surface markers are used to characterize ES cells. Examples are SSEA-4, TRA 1-60, and TRA 1-81 that were originally identified in embryonal carcinoma cells and human pre-implantation embryos [98]. Similarly, a number of transcription factors that play important roles in maintaining self-renewal, are currently used to characterize isolated ES cells from blastocysts. For example, the transcription factor Oct3/4 [99 100], Sox2 [101] and Nanog [102]. Isolation of ES cells from human embryos has tremendous potential in clinical applications, however ethical concerns have to be taken into account.

Induced Pluripotent Stem Cells

Pioneering work by John Gurdon back in the 1960s demonstrated the cloning potential by transferring a nucleus from a somatic cell of a tadpole into an enucleated egg [103]. It was not until 2006, that Shinya Yamanaka and colleagues made the discovery that four factors, Oct4, Sox2, Klf4 and c-Myc (OSKM), when ectopically introduced, were capable of reprogramming a somatic mouse cell into a state of pluripotency [104]. One year later they were able to confirm

this for human cells [105]. Yamanaka's research demonstrated that fully differentiated and matured cells retain the potential which resembles the embryonic stage. This groundbreaking discovery, together with Gurdon's work, was awarded a Nobel Prize in 2012. Their research demonstrates an indefinite potential for disease modeling and regenerative medicine. Similar to ES cells, iPS cells express markers of an undifferentiated state and possess the capacity for self-renewal and differentiation to any cell of all three germ layers. Human ES cells are bound to multiple ethical regulations due to the destruction of the embryos whereas iPS cells offer a clear ethical advantage, representing an unlimited source of any cell type of interest [106].

1.2.3 REPROGRAMMING, CULTURE CONDITIONS AND CHARACTERIZATION OF IPS CELLS

Cellular reprogramming occurs when a somatic cell resets back to the pluripotent stem cell state. During recent years, various methodologies have been developed to achieve this. Here, we focus on reprogramming via ectopic expression of defined transcription factors, therefore even though somatic cell nuclear transfer, cell fusion and direct reprogramming are also approaches that are or have been used, they will not be the focus in this thesis but is reviewed in [107]. It is important to note that reprogramming is achieved differently depending on the cell source, usually attributed to differences in endogenous levels of reprogramming factors (Figure 8, left), as well as the delivery method of choice (Figure 8, right) [108].

Summary of reprogramming methods

Ectopic expression of OSKM in somatic cells promotes their transition to a pluripotent state. Takahashi and Yamanaka used retroviral vectors in order to reprogram mouse and human fibroblasts [104, 105]. However, nowadays different methods are used to generate iPS cells. These techniques include integrative and non-integrative methods that allow for different efficiency and safety of reprogramming. **Integrative methods** utilizing retro- and lentiviral constructs involve integration of the genetic material into the host genome. Retroviruses were used in the original studies conducted by Yamanaka and offer an efficient and easy form of delivery. In the same way, lentivirus integrates into the host genome. However, their capability of integration in the host genome comes with risk of mutagenesis [109]. Other risks of the two viral methods include the reactivation of transgenes with the implication of off-target effects. The transfection with plasmid or linear DNA and transposons, presents an alternative to viral vectors, although quite inefficient. On the other hand, **non-integrative methods** overcome the safety risks mentioned above, thus being a preferable choice. Some of these techniques are DNA based methods such as adeno- or RNA based viruses as the often used Sendai virus (SeV). However, adenoviruses are labor intensive to produce and require multiple infections. In contrast, SeV, a negative-strand single stranded RNA virus, is an efficient expression method that expresses the transgene without host genome integration. Of note, the viral genome remains in the host cell for a period of time due to which extended passaging is required [110]. Episomal vectors are extrachromosomal DNA that replicates independently from the host DNA. They are compared to viral vectors a low-cost solution. Their disadvantages are the relative inefficiency, and the requirement of serial transfections.

Last but not least, mRNAs transcribing the four factors OSKM and Lin28 are able to reprogram a somatic cell via transfection methods. The reprogramming mRNA is modified that reduces the immunogenicity of this technique [111]. All in all, the reprogramming method is of choice and mainly depends on the application and purpose of the desired cells (Figure 8, Right panel).

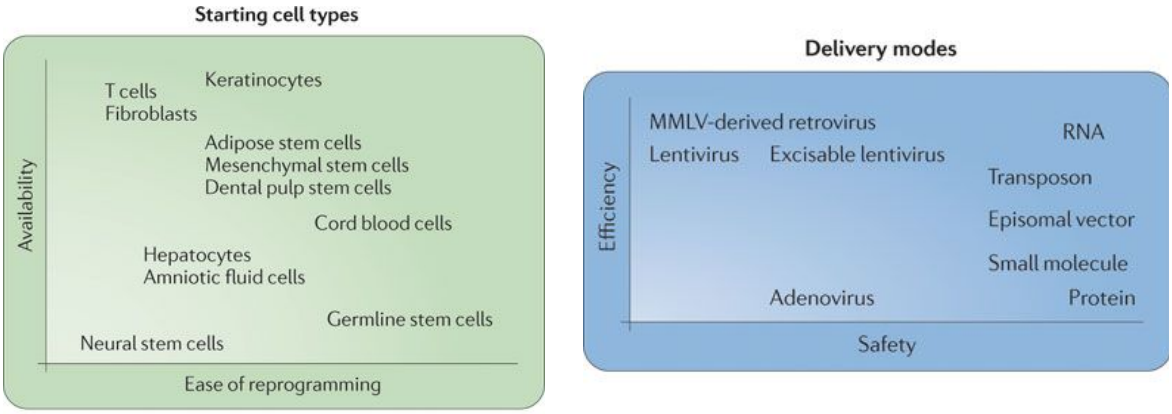

Figure 8. Reprogramming Strategies. By González et al, 2011, with permission of the publisher, Nature.

Nuclear reprogramming by defined transcription factors (OSKM)

Whereas, Oct4 [112], Sox2, and Nanog are key regulators of embryonic development and expressed in the ICM of the blastocyst, Nanog is not essential for the reprogramming process [104, 113]. Klf4 has key roles in development, proliferation and differentiation and is expressed in ES cells. C-Myc is a proto-oncogene and among other functions linked to proliferation, differentiation and apoptosis. Moreover, it is associated with acetyltransferase complexes allowing the chromatin to relax. Thereby, it facilitates Oct4 and Sox2 binding to their respective loci and drive the expression of target genes which induces reprogramming. Oct4, Sox2, and Nanog cooperate in order to maintain expression in an auto-regulatory manner. High expression of c-Myc can lead to increased levels of p53 which might potentially promote apoptosis during reprogramming. However, Klf4 has been shown to block p53 thus subsequently allowing reprogramming [114, 115]. Collectively, downregulation of p53 has been shown to increase reprogramming efficiency [116].

The process of reprogramming consists of two phases, the stochastic phase (**phase 1**), which is the initial phase and the hierarchical phase (**phase 2**). During phase 1, lineage-specific genes are downregulated, probably due to direct effects of OSKM and subsequent upregulation of pluripotent markers. Next, remodeling of the chromatin occurs with activation of endogenous pluripotent genes [117]. Initiation of phase 2 begins when Oct4, Sox2, and Nanog are able to be expressed endogenously and thereby reactivate the auto-regulatory loop that leads to restoration of the pluripotency network. This process includes self-maintaining of pluripotency genes and the reactivation of telomerase. Notably, epigenetic changes take place during reprogramming and are important for iPS cells properties [118]. Somatic cells contain condensed heterochromatin, compared to iPS cells which has a more accessible and open chromatin conformation. Moreover, activating histone marks and

hypomethylation of DNA for pluripotency genes are some of the processes that are involved in the reprogramming and maintenance of pluripotency [119].

Culture conditions

Optimization of cell culture conditions for iPS cells are crucial to increase safety of production, generate homogenous cultures of iPS cells, and for final clinical application purposes. The main goal during the past decade has been the development of chemically defined, and non-animal derived components (xeno-free) to derive and grow iPS cells for regenerative medicine and drug discovery. This allowed for a better standardization of protocols, avoidance of spontaneous differentiation, and heterogeneity of expanded cells [120]. Therefore, cell culture conditions are crucial for the maintenance of high quality iPS cells including their pluripotency features and differentiation potential. Briefly, ***in vitro*** iPS cell culture need (i) **growth media**, (ii) **extracellular matrices** and (iii) **environmental factors**.

(i) As mentioned peviously, the ultimate goal for therapeutic use of iPS cells is to develop serum-free, xeno-free, and chemically defined media. The first medium used to culture pluripotent stem cells contained fetal bovine serum (FBS) and cells were cultured on Mouse embryonic fibroblasts (MEFs) also refered to as feeder cells. MEFs are responisble of secreting growth factors needed for the mantaince of the co-cultured iPS cells. Today, media are more standarized and better defined as a result of replacing most of the xenogeneic elements[121, 122]. Thus, defined medias contain protein components from human or recombinant origin that allows for feeder-free conditions (see (ii) for feeder-free conditions) [123].

(ii) Pluripotent stem cells including ES cells and iPS cells, grown ***in vitro*** as monoculture are in need of supporting factors. Typically pluripotent stem cells used to be cultured on mitotically inactivated MEFs [93]. MEFs secrete growth factors that support iPS cell in culture. However, MEFs can be a source of viral and bacterial contamination. Of note, there is requirement of animal products such as FBS supplemented medium for MEF cultivation. In addition, laborious preparation is required for their mantainance. For these reasons, large production of iPS cells is not feasible in culture conditions based on MEFs. Feeder-free systems have been developed based on components of the extracellular matrix (ECM). Indeed, in absence of feeder cells, factors that promote survival and proliferation are still a requisite. Currently, matrixes such as Matrigel [TM] are utilized to maintain pluripotent stem cells. Matrigel is extracted from Engelbreth-Holm-Swarm mouse tumor and contains a mixture of ECM components; fibronectin, collagens, and laminins in addition to various growth factors. Despite its ease of use, batch to batch variations and animal derived components display a disadvantage depending on the final application of the cells. Alternatives are recombinant fibronectin or vitronectin which are part of the ECM and are rich in arginine glycine aspartate peptide sequences, required for cell-cell adhesion [124]. Laminins are glycoproteins present in the ECM and constitute the basement membrane. Furthermore, laminins are expressed in human ES cells [125]. Recently,

human recombinant laminins have been reported to maintain long term self-renewal of pluripotent stem cells and allow for clonal derivation and survival [126, 127]. Cell culture conditions are extremely important not solely for maintaining pluripotent characteristics but also to retain genomic stability [128]. Importantly, it has been shown that cell culture conditions such as media, passaging methods, and supplements can induce mutations for example in p53 [129], an important regulator of genomic stability in the cells.

(iii) Enviromental cues include physical and phyisiological cues that iPS cells are subjected to when grown *in vitro*. Examples of environmental cues are passaging, cryopreservation, humidity, oxygen levels, and temperature.

Characterization of pluripotency

One requirement after generation of iPS cells is the validation of the cell potential in terms of pluripotency and differentiation. A diverse range of methods are used to assess pluripotency, most commonly morphology, expression of surface markers, and transcription factors that indicate an immature state and assess differentiation potential. Typical surface markers are SSEA-4, TRA-1-60 and TRA-1-81 which can be analyzed using simple molecular techniques such as immunocytochemistry or flow cytometry. The transcription factor OCT4 is expressed in pluripotent cell populations both *in vivo* and *in vitro* and its downregulation takes place during differentiation [112 99]. Similarly, SOX2 is essential to maintain cell pluripotency and self - renewal [130] whereas NANOG cooperates with SOX2 and OCT4 to maintain pluripotent properties [131]. Gene expression profiling is essential to determine molecular signatures that are unique for ES and iPS cells. For example, bioinformatics tools such as the PluriTest have been a step forward for the characterization of pluripotent stem cell lines. This is an easy-use bioinformatic tool based on gene expression profiles of around 300 pluripotent stem cell lines[132] which allows for a good indication of the cell line quality. However, the expression of pluripotent markers is not exclusively important to assess the full pluripotency potential of the reprogrammed cells. Due to the possibility of clonal selection occurring during cell culture techniques and high proliferation rates, *in vitro* iPS cells need to be routinely checked for genomic integrity [128]. To assess differentiation potential in human pluripotent stem cells, teratoma formation has been widely used. To check for teratoma formation iPS cells are injected into immunocompromised mice and the resulting teratoma is validated for the presence or absence of the three germ layers. Similarly, markers for the three germ layers can be validated in vitro after generation of embryoid bodies (EB). Since epigenetic phenomena are a hallmark during reprogramming as well, studies of the epigenetic landscape of iPS cells can be used as a tool for characterization.

1.2.4 DISEASE MODELING WITH IPS CELLS

Disease modeling

The development of iPS cells opened up new avenues for disease modeling, drug screening and discovery, and regenerative medicine including cell transplantation therapies. This

thesis focuses on disease modeling as one application for iPS cells. Most of the current systems used to study cancer rely on murine model organisms and *in vitro* systems of immortalized or primary cell cultures. Despite the usefulness of these models, interspecies differences cannot be ignored, such as differences in disease progression and drug response [133]. In addition, prolonged culture of cancer cells can alter their characteristics compared to the tumor source. It has been shown that only 5 % of tumor associated genes were expressed in the cell lines when compared to the original tumor [134].

iPS cell technology provides great advantages due to the ease of cell generation from any individual in context with its own genetic background. One of the most attractive features of iPS cells is their representation as an indefinite source of disease-relevant cell types. Patient-derived iPS cells demonstrated potential to uncover mechanisms that have been unknown or poorly understood in humans. Moreover, iPS cells provide disease-relevant cell types useful for drug screenings [135].

Modeling disease with cancer-predisposing genes

Complex diseases with sporadic origin and late onset have been successfully modeled using iPS cell technology [136, 137, 138]. This also applies to modeling diseases such as cancer [139]. The first reports involved the establishment of iPS cells from a diverse range of cancer cells as proof of principle [140, 141, 142]. Pancreatic ductal adenocarcinoma (PDAC) lacks models to understand early stages of the disease [143]. A study showed that iPS cells derived from pancreatic cancer cells could recapitulate the early stages of PDAC. In addition, the model was used to identify novel targets for the diagnosis and treatment of PDAC [143] (Figure 9a). However, due to the genomic and epigenetic complexity of cancer cell lines, the reprogramming process of cancer cells has been challenging. Although some studies have shown that pluripotency can pause cancer progression and restore differentiation *in vitro* [144], others tested the effect of reprogramming *in vivo*. Partial reprogramming *in vivo* did not lead to teratomas but severe tissue dysplasias[145]. Therefore, other approaches can be used in order to not solely model the disease but understand the contribution of certain alterations in cancer development. Germline mutations are rare, however 43% of cancer predisposition genes with germline mutations overlap with mutations found in sporadic tumors [146]. Lee and colleagues, reprogramed fibroblasts from Li-Fraumeni syndrome patients (LFS) that carry hereditary mutations in TP53. These mutations led to the onset of site-specific cancers including osteosarcoma, breast cancer, and brain tumors [147]. Because murine models do not fully recapitulate the tumor spectrum of these patients and p53 is usually linked to other oncogenic drivers, other models were needed to decipher the role of p53 in cancer development. LFS patients' healthy fibroblasts were reprogrammed into iPS cells and differentiated into osteoblasts with successful recapitulation of osteosarcoma lead to the identification of regulatory mechanisms which bears the potential for clinical implications [147]. Therefore, many different patients could potentially benefit using this same approach (Figure 9b).

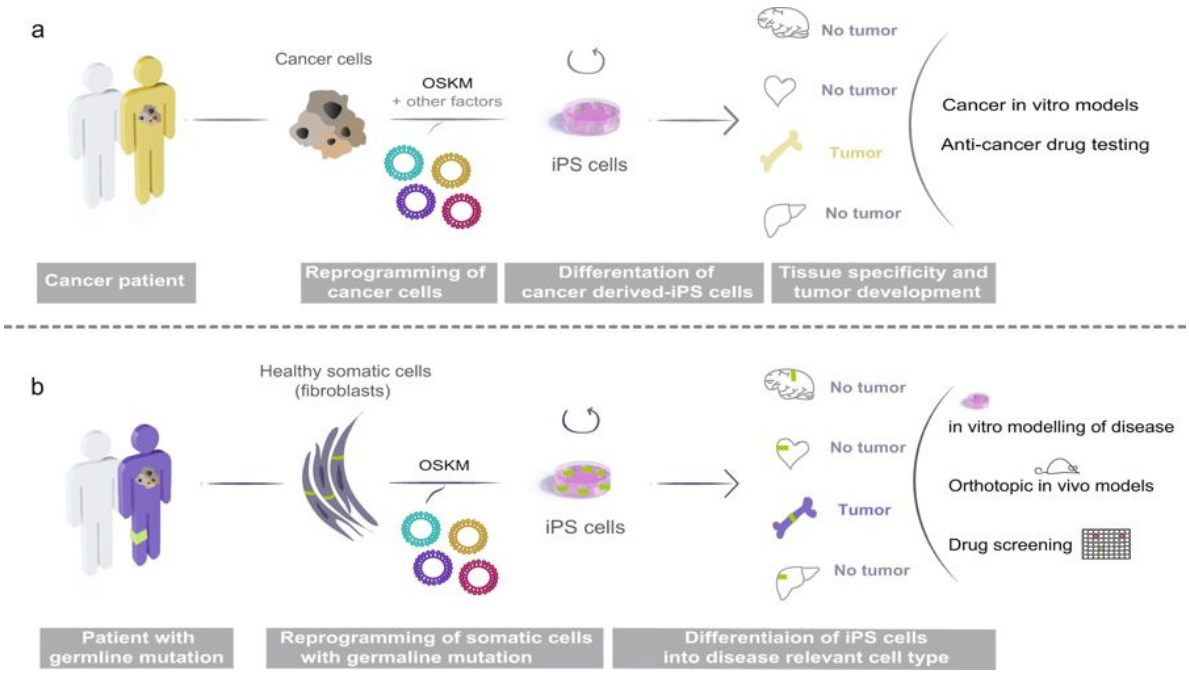

Figure 9. Approaches to model cancer using patient-derived iPS cells. Cancer cells from the patient can be reprogrammed to be used for establishing new models and drug screening platforms (a). Somatic cells carrying germline mutations in a cancer patient are used to generate iPS cells that in turn are used for disease modeling and drug testing in vitro and in vivo. Marin Navarro et al, 2018, Cell Death Discovery, printed with permission from the publisher.

Here, the focus will be on using non-cancerous cells for reprogramming albeit carrying germline mutations implicated in the development of cancer. We have established models that allow monitoring of tumor formation from the first genetic hit.

(i) Hereditary Neuroblastoma

PHOX2B and ALK mutations have been found in hereditery NB [148] being perfect candidates to establish iPS derived models. Germline mutations in NB are rare, however as previously described in section 1.1.3.4 [24], they are found sporadically and can thus be used to model NB using patient-derived iPS cells.

(ii) Inherited Medulloblastoma-associated syndromes

Germline mutations in PTCH1 are linked to Gorlin syndrome, which is a predisposition to basal cell carcinomas, congenital abnormalities, and MB. Other mutations in genes such as SUFU and APC can be associated to different syndromes predisposing to MB and other cancers. Therefore, using iPS cells derived from patients carrying these germline mutations can be benefitial to understand not only MB but a broad range of other related diseases.

1.2.5 MAKING THE RIGHT PROGENITOR FOR CANCER MODELING

Modeling cancer using patient-derived iPS cells undoubtedly opened new insights in basic research and unraveled new potential clinical targets. The ***repertoire*** of cell types that can be derived from iPS cells is immense. However, generating the right progenitor or relevant cell type to best mimic disease is most important. Identifying the most relevant cell type for every

disease we would like to model, is as crucial as the validation of pluripotency of iPS cells in the first place. After identifying the cell type of interest, there are a few aspects to take in consideration such as; available differentiation protocols, efficiency of the protocol, homogeneity and quality of the resulting cell population, and maintenance and characterization of the cultured cells. In this thesis, we aimed to model two different childhood cancers, NB and MB. In order to do that, we first required to generate what is thought to be the cell of origin of each respective disease. Therefore, I revise two different progenitors that can be differentiated from iPS cells; Neural Stem Cells (NSC) and NCC, in chronological order during development.

1.2.5.1 NEURAL STEM CELLS FROM IPS CELLS

Neurulation during development

Neurulation in vertebrates is the formation of the neural tube which gives rise to the central nervous system; the brain and the spinal cord. For this to happen a mesodermal structure, the notochord, needs to be formed and acts as the major axis in the body after the three germ layers are generated. The notochord will orchestrate the ectoderm to become a thick flat plate of cells that we commonly name the neural plate. Importantly, this plate is composed by a single cell layer that forms the neuroepithelium. The neural tube extends from the rostral-caudal axis which bends in a tube shape known as the neural tube. The neural tube sits just underneath the ectoderm. During a process in which the neural plate borders fuse to each other NCC emerge and migrate out to other parts of the embryo (Figure 10).

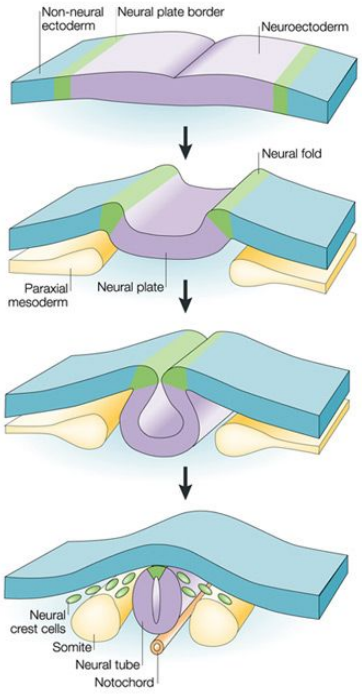

Figure 10. Neurulation process. The neural plate border (green) and in between, the neuroectoderm (blue). During the process of neurulation, the neural plate borders form neural folds which elevate and fuse with each other resulting in the formation of the neural tube. NCC will delaminate from the dorsal part of the neural tube. Gammil & Bronner-Faser, with permission of the publisher, Nature 2003.

Neuroepithelial stem (NES) cells and Neurogenesis

Neuroepithelial stem (NES) cells form the neuroepithelium present in the neural plate during neurulation.These represents the primary neural stem cells (NSC) which can divide symmetrically, giving rise to two identical daughter cells (self-renewal), or asymmetrically, which generates an identical daughter cell plus a second more specialized cell type, in this case, a radial glia (RG) cell or a neuron type [149]. RG which are more restricted than NES cells, display astroglial properties and generate most of the neurons in the brain. This differentiation process from NSC to neurons is called neurogenesis [150]. Consequently, NSC are in charge of forming all neurons from CNS.

NSC from iPS cells

Understanding human neurogenesis and brain development is crucial to elucidate CNS related diseases such as neurodegenerative and neuropsychiatric disorders, and brain tumors. Therefore, the scientific community has put a lot of effort trying to understand NSC function and their differentiation. The possibility of obtaining unlimited neural stem cell progenitors ***in vitro*** from pluripotent stem cells has been a major advance for disease modeling and regenerative medicine. Neural induction has been extensively studied in Xenopus and chicken embryos. Mechanisms underlying neuroectoderm differentiation involve BMP, WNT, and FGF signaling pathways[151]. The first protocols addressing pluripotent stem cells were based on EB formation. Usually performed in the presence of serum or serum replacement with formation of neural rosettes, a morphology indicative of the neuroepithelium [152, 153, 154]. In 2009, Chambers et al, established an optimized protocol targeting both branches of the SMAD signaling pathway by using Noggin, a BMP inhibitor, and SB431542, a TGF-β/Activin/NODAL pathway inhibitor, which block BMP and TGF-β pathways that utilize SMADs as transducers. This allowed for the exit of cells from the pluripotent state while blocking the formation of mesoderm and non-neural ectorderm achieving higher efficiency of NSC [155]. In presence of FGF and EGF, NSC can be propagated ***in vitro*** [154, 156].

1.2.5.2 GENERATING HUMAN NEURAL CREST CELLS

Signaling pathways during NCC development

The complex nature of NCC during embryogenesis has been reviewed in section 1.1.3.3. NCC multipotency leads to the generation of a wide spectrum of cell types that constitutes the body (Figure 11). Therefore, it is not surprising that deregulation during NCC development implicates various human diseases but also a promising potential for clinical applications [157]. Most of our understanding in NCC biology has come from chicken embryos, zebrafish and mouse. NCC emerge at the border of the neural plate (Section below, Figure 10 and 11) and the non-neural ectoderm that will later form the epidermis. When the neural plate folds (Figure 10), the neural folds from each side of the ectoderm come together and fuses (Figure 10). During the neural tube closure, NCC migrate out colonize the embryo and differentiate (Figure 11). The regulatory gene network that governs and regulates NCC formation and differentiation is extremely complex [158]. It is currently understood that NCC induction occurs during early gastrulation mediated by BMP, WNT and FGF signaling

pathways [159]. First evidences in Xenopus showed the importance of BMP signaling pathway during NCC formation. The growth factor BMP4 was produced prior to neural induction inhibiting neural tissue formation and instead promotes the formation of the epidermis. Antagonists of BMP block this process in order for neural induction to occur [160]. However, later studies showed discrepancies on how this process take place and several models have been proposed [161, 162]. Nevertheless, it was established that intermediate levels of BMP are required but not sufficient for NCC induction. Indeed, the cooperation of other signaling pathways is necessary for NCC formation [158]. For example, members of the WNT family and FGF signaling pathways have been shown to play a role in NCC development[163, 164].

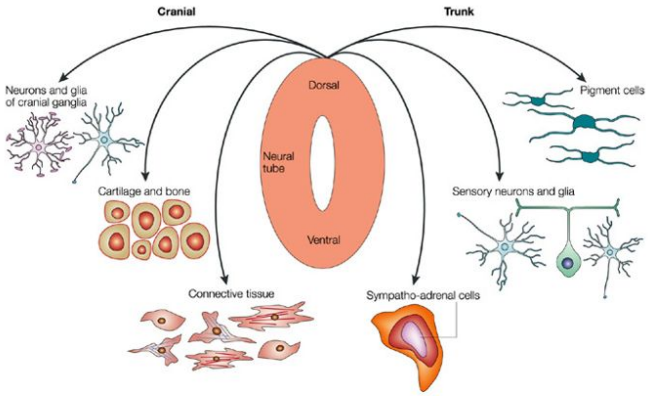

Figure 11. Derivatives of NCC. NCC migrate out from the dorsal neural tube after its closure. With permission from the publisher. Knecht & Bronner-Faser, Nature Reviews, 2002.

NCC derived from iPS cells

Most protocols for the generation of NCC from pluripotent stem cells rely on the understanding of NCC biology [158] and studies in which human pluripotent stem cells could be differentiated into neuroectoderm [155, 165]. Lee and colleagues showed the presence of NCC at the neural rosettes stage by co-culturing ES cells on stromal cells. However, the use of co-culture systems is inefficient and heterogeneous, thus not ideal for most of applications. In 2010, the same group improved the protocol developing a feeder-free system using the SMAD dual inhibition strategy (also see section above, 1.2.4.1). Therefore, defined medium containing Noggin (BMP inhibitor) and SB431542 (TGF-β/Activin/NODAL pathway inhibitor) was used [165]. Despite the inefficiency of the protocol, the combination of these two factors and culture conditions was a major breakthrough that initiated a new era of NCC generation protocols. In 2011, Menendez et al [166] combined WNT pathway activation by inhibiting GSK3, a WNT antagonist and the suppression of TGF-β/Activin/NODAL pathway. In this study, it was observed that BMP inhibition was not required for NCC generation [167]. In 2013, Mica and colleagues reported that an initial pulse of BMP inhibition and TGF-β/Activin/NODAL pathways followed by WNT activation and withdrawal of the SMAD dual inhibition strategy increased efficiency of NCC generation. Collectively based on the mentioned studies, it becomes clear that the effect of BMP signaling during NCC generation from human pluripotent stem cells still remains unclear

1.2.5.3 BRAIN ORGANOIDS

Organoids derived from pluripotent stem cells

Until now, the relevance of 2D systems has been discussed. However, ***in vitro*** 3D systems have shown to be structurally and physiologically more similar to the recapitulation of an ***in vivo*** situation. 3D ***in vitro*** systems display higher complexity, are functionally and structurally

more representative of cellular organizations [168, 169]. Already, in 1965 cell dissociation and cellular aggregation was used to describe an organoid in developmental biology studies. Today, this concept has developed further and a new definition of organoids has been created; 3D structures that originate from stem cells and are constituted of tissue-specific cell types that spatially organize [170]. Pluripotent stem cells have been used to generate many different organoids like brain, gut, kidney and retina [170]. Therefore, the generation of patient-specific organoids from iPS cells holds a promising step forward in the comprehension of disease modeling, drug testing, and gene therapy.

The human brain

The brain is the most complex of all organs of the animal kingdom. To understand brain development, animal models have been extensively used and thus are the basis of most of our knowledge about the brain. Specific features and functional studies in human brain have been completely limited to post-mortem observations. Thus, scientists have been in need to establish ***in vitro*** models close to ***in vivo*** brain development. Whereas two-dimensional cell culture has been vital for the study of the brain, it is clear that the recapitulation of complex and diverse cell structures is limited. 2D systems can be quite homogenous, with presence of non-solely neural tissue that is far from the ***in vivo*** situation.

As previously described, the brain develops from the neuroepithelium, more specifically from a sheet of NES cells in the neural plate. During brain development, the ventricles are formed after the neural tube closure. Adjacent to the ventricles, the NES cell layer is present and its major function is to proliferate with the purpose to increase and expand the number of progenitors before neurogenesis commences. This proliferative zone is named the ventricular zone (VZ). During the division of progenitors, a process named interkinetic nuclear migration (INM) takes place which consists of the movement of NES cell nuclei from the basal to the apical side of the neuroepithelium [171]. This is a typical process within the epithelium and it is essential for the cells to find the right position and initiate mitosis during entire neurogenesis. Right before neurons are born, NES cells change their biological properties and become RG cells [172]. Recently, it has been shown that RG differ in between different niches. In a vertical plane, RG cells continue to divide symmetrically but additionally asymmetrically giving rise to either another progenitor or a neuron. In humans, the beginning of neurogenesis initiates during week 5 and 6 of gestation. Importantly, RG will establish another neurogenic niche, the subventricular zone (SVZ) that is different from the VZ because of a different cell orientation and marker expression profile [173]. At that particular stage RG are named intermediate progenitors or basal progenitors that divide symmetrically giving raise two neurons each time [174]. The expansion of the neocortex occurs according to this process of divisions, being generated from different progenitor and neurons that migrate basally and organize themselves in layers. While the deepest layers of the cortex will contain the oldest neurons, the newest neurons will migrate to the most outer part of the cortex. Together, the fully formed neocortex contains 6 neuronal layers [175].

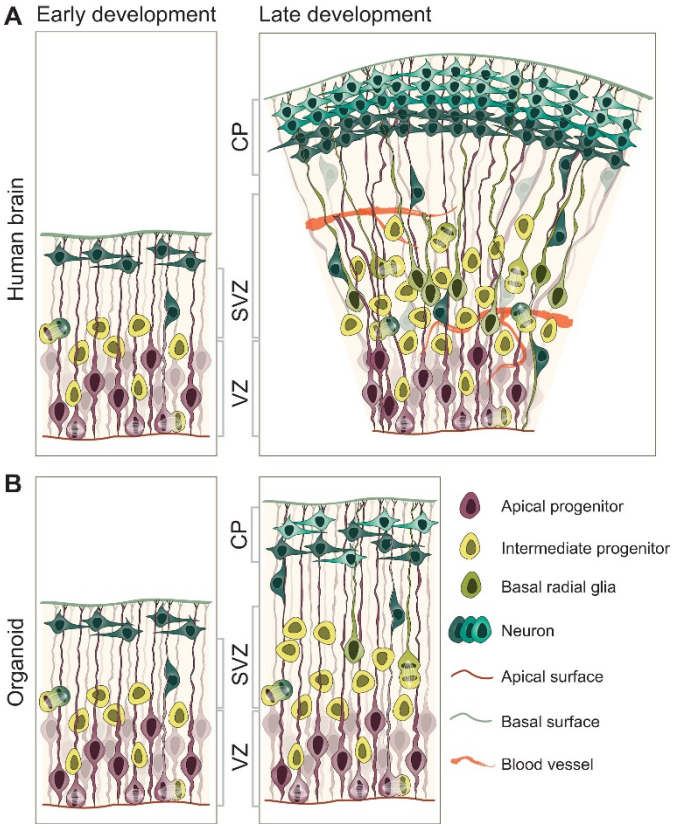

Figure 12. *In vivo* versus *in vitro* human brain. (Top) The human brain in early and late development. (Bottom) Representation of the organoid during early development, similar to human and late development which shows structural and cellular differences.

Brain organoids from iPS cells

The cellular biological features of all the various cell types contributing to human brain development are extremely complex and heterogeneous, even though it has been simplified here, a more detailed overview can be revised in several reviews [175, 176]. *In vitro* establishment of neural progenitors has shown to be capable of recapitulating and differentiating into a diverse range of specific neurons including layer specification [177]. However, considering the complexity of an organ such as the human brain, 3D culture methods represent a great advantage in the field.

Based on the notion that ES cells spontaneously differentiate into neural fate without the presence of inhibitors [178], Sasai improved culture of 3D methods using floating EB in serum-free conditions (SFEB) [179]. This, included reaggregation of cells and subsequent seeding on coated dishes. With the addition of specific signals and when plated, forebrain progenitors were generated (SFEBq) [180]. Mariani et al, showed that modification of this protocol allowed for a fair recapitulation of the developing forebrain [181]. Although it was clear that neural induction did not require help of serum or other morphogens, the efficiency of the protocols had to be optimized. As explained in section 1.2.4.1, germ layer specification depends on several signaling pathways such as TGF-β that includes SMADs as downstream effectors. With this, Chambers et al, engineered a combination of factors that promoted differentiation of neural progenitors from pluripotent stem cells overcoming the need of using EB formation [155]. A consequence of this was a blooming era for neural differentiation from pluripotent stem cells. From this point of view, 3D organoids, as we know them today were just an emerging era at that particular time. Hans Clevers demonstrated that intestinal stem cells could

generate tissues in 3D structures that resembled the special structure of the intestinal crypt and epithelium [182]. The use of ECM components was a relevant difference compared to other protocols. ECM supported growth and spatial structure of the epithelia, which organized using a basal-apical orientation. In the same manner, many other epithelial organs have been modeled such as stomach [183], lung [184] and esophagus [185]. The breakthrough that ECM, concretely matrigel, was supportive of epithelial structures was an important step forward for the development of brain organoids as well. Madeline Lancaster generated brain organoids through a combination technique that includes the embedding of EBs in matrigel. Importantly, these organoids present a different variety of cellular identities including hind-, mid- and forebrain cells thus referred to as whole-brain organoids [168]. On a cellular level, it is incredible to see the resemblance of the ***in vivo*** early developing human brain with the 3D cultured organoids. Figure 12 represents the structural and cellular similarities of early stages of brain development compared to 3D brain organoids (a and b, left panel) whereas in later stages present a bigger difference. Figure 13 shows the transcriptomic resemblances between an **in vivo** human developing brain and a 3D brain organoid.

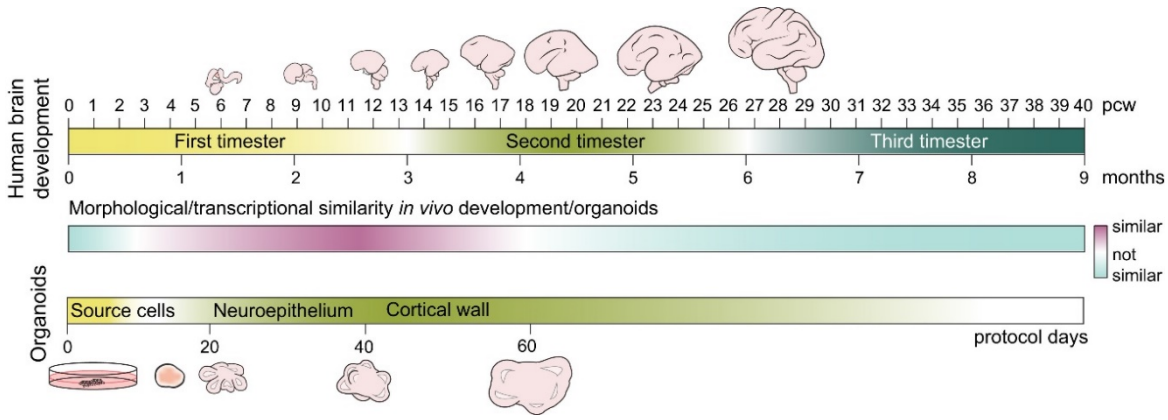

Figure 13. Timeline of the human developing brain. Purple indicates similarity between ***in vivo*** and ***in vitro*** systems based on cell-biological transcriptomic data of different studies. pcw – post-conception weeks[186]. With permission of Elsevier, Kelava et al, 2016, Cell Stem Cell.

1.3 TP53, THE GUARDIAN OF IT ALL

p53 is a transcription factor belonging to the p53 family, which also includes the transcription factor members p63 and p73 [187]. This family is evolutionary conserved which means that its origin traces back over a billion years ago. Thus, all members of the family share structural homologies. Of note, in humans each of these genes is located on a different chromosome. p53 for example, is situated on 17p13.1, p63 on 3q27-29, and p73 is located on chromosome 1p36.3. Even though they share similar structures, they do not necessarily exert the same functions [188] . However, in this thesis only p53 is reviewed.

1.3.1 STRUCTURE, FUNCTION AND REGULATION

Structure of the p53 protein

The p53 gene encodes for a 393 amino acid protein that forms a homotetramer. The N-terminal part of the protein contains two distinct regions; a transactivation domain (TAD) and

a proline-high region. The TAD region is especially important for protein-protein interactions for example with MDM2, a negative regulator of p53. The core of the protein constitutes the structure for a DNA binding domain (DBD), an immunoglobulin-like-β-sandwich that acts as a scaffold for DNA, followed by the oligomerization domain (OD) permitting for the tetramerization of the protein. Last but not least, the c-terminal domain (CTD) undergoes posttranslational modifications such as ubiquitination and phosphorylation that leads to different regulations and functionalities of this protein [189]. Most of p53 research refers to the full length protein, however up to twelve isoforms are encoded from the same gene, with and without presence of the TA domain [190].

Versatile functions of p53

p53 is a transcription factor that is activated as a result of many cellular stresses such as DNA damage, oxidative stress, and oncogene activation among others. The main role of p53 is to protect the cell by attempting DNA repair, blocking cell cycle or inducing apoptosis [191]. One of the most well-known effectors of p53 is p21, which blocks cell cycle by interacting with cyclin-dependent kinase complexes, preventing phosphorylation of RB proteins and inactivating E2F-dependent transcription [192]. Importantly, p53 regulates DNA repair and genomic stability. One of the mechanisms that p53 induces upon DNA repair is apoptosis by the activating the transcription of pro-apoptotic genes. For instance, the p53 target BAX inhibits the pro-survival family bcl-2 [193], or the p53 upregulated modulator of apoptosis (PUMA) can bind directly to Bcl-2 leading to cytochrome-c release from mitochondria [194]. On the other hand, p53 controls genomic stability by regulating centrosome duplication [195]. Another function regulated by p53 are metabolic pathways such as glycolysis and oxidative phosphorylation [196] with further implications in proliferation and cell fate determination. Moreover, cellular reprograming is antagonized by p53. In line with this, OCT4 and KLF4 repress p53 transcription [197]. However, at a cellular level, p53 controls many distinct functions by exerting its transcriptional activity of many genes (Figure 14).

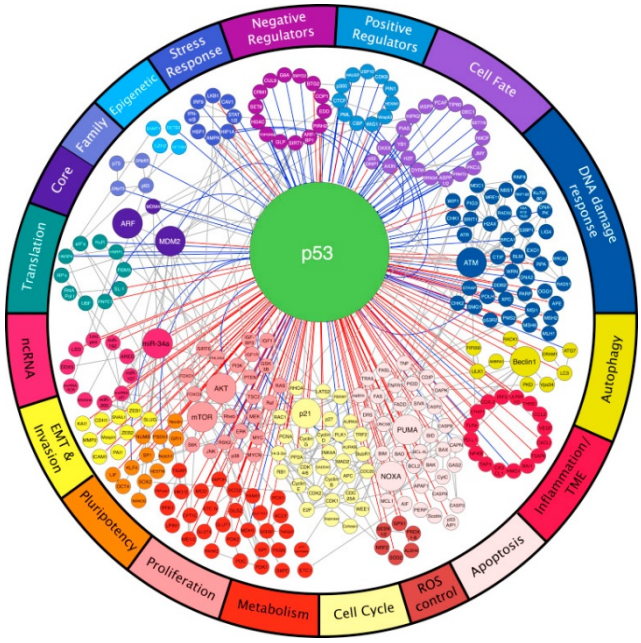

Figure 14. Network of regulators, functions and target genes of p53. Kustenhuber and Lowe et al, 2017, with permission of the publisher, Cell.

1.3.1.1 TP53 DURING DEVELOPMENT

p53 is a well-known tumor suppressor thus, playing an important part in cancer development. Mutations leading to inactivation of p53 are shown to be one of the most common events in adult human cancers. Unfortunately, p53 loss is usually associated with poor prognosis [198].

However, since p53 regulates such vast range of functions, mostly tumor suppressor functions, whether p53 is important during early brain development is still poorly understood. In normal cells, p53 levels are low and is not until a stress-induced signals occurs that p53 can be released from regulators such as Mdm2. Mdm2 is an E3-ubituitin ligase that flags p53 for proteosomal degradation [199]. Mdm2 KO mice are lethal by early embryonic stage during implantation, and this phenotype is rescued by depletion of p53 indicating the importance of negative regulation of p53. An elegant study showed that p53 knocked in mutant mice revealed lethality at E10.5 and between E13.5-15.5 varying on the p53 levels. In this study, it was described that unappropriated activation of p53 led to several developmental abnormalities including; brain, heart and bone/cartilage defects [200]. When looking into the mouse system, p53 is highly expressed during development, therefore it was surprising that the first study reporting p53 KO mice did not show developmental problems, yet quick tumor burden was observed [201]. Later, two different groups showed that p53 KO mice exhibit exencephaly, a neural tube disorder, as a major developmental problem in p53 KO mice. Nevertheless, this phenotype was varied depending on genetic background and gender of the mice [202, 203].

p53, brain development and stem cells

At embryonic day 10.5 (E10.5), p53 mRNA is shown to be expressed ubiquitously throughout the whole brain [204]. At the time that differentiation and specification occur, p53 expression becomes limited to the cortical plate [205]. Transgenic mice expressing ***lacZ*** under a p53 promoter confirmed high transactivation of p53 during E10.5 [206], specifically between the midbrain region of the brain and the cerebellum. In newborn mice, p53 is localized in cerebral cortex regions, the surface of the thalamus, cerebellum, and hippocampus and exerts sporadic activity [206, 207]. Moreover, Mdm2 conditional mouse models demonstrated that loss of Mdm2 during CNS development exhibited massive p53 dependent apoptosis leading to degeneration of the neuroepithelium and the cortex [208]. Similar to the human brain, the mouse brain develops from NSCs during embryogenesis. Some studies have identified the presence of p53 in neurogenic niches such as the SVZ [209]. Neurosphere ***in vitro*** assays from p53 null mice demonstrated higher capability of self-renewal and less apoptosis [209, 210]. It has been shown that p53 inhibits the BMP-SMAD pathway assessing the differentiation potential of NSCs from $p53^{-/-}$ mice. BMP-SMAD inhibition suggests that loss of p53 increases proliferation and self-renewal of neurospheres and promotes neural differentiation [211]. Of note, all studies performed so far have been done in mice hence the role of p53 during human neurogenesis remains unclear.

2 AIMS

The general aims of this thesis were (i) to develop *in vitro* and *in vivo* models using patient-derived IPS cells to mimic the early developmental malignancies Neuroblastoma (NB) and Medulloblastoma (MB). (ii) To study the role of p53 during human brain development. The aims are further divided into sub-ordinate aims:

(i)

I. To generate patient-derived iPS cells from two NB patients
II. To establish a robust protocol for the generation of NCC from iPS cells
III. To model ALK driven-NB using patient-derived iPS cells *in vivo*
IV. To model SHH-MB using Gorlin patient-derived iPS cells

(ii)

V. To establish human *in vitro* models to study p53 during brain development

3 RESULTS AND DISCUSSION

3.1 A MODEL FOR ALK-DRIVEN NB USING PATIENT-IPS CELLS (PAPER I, II, AND III)

iPS cells and their derivatives mimic early stages of human development, which makes them an attractive model to study early onset diseases such as childhood cancers, which are thought to originate in stem or progenitor cells [139]. Therefore, in paper I and paper II, the resources necessary to model childhood cancers were created [212, 213]. In paper III we use the resource created to model NB development by orthotopically injecting patient-derived NCC in the adrenal gland of immunocompetent mice.

NB is an embryonic childhood cancer that, despite being rare, presents a high mortality rate in infants. The adrenal gland, derived during PNS development, is one of the most common locations for primary NB tumors. We established a patient-derived iPS cell model to study initiation and progression of NB. Gain-of-function mutations of ALK are a characteristic of NB [35]. ALK mutations can be both hereditary or somatically introduced. To further evaluate whether NCC from NB patients carrying a germline mutation in ALK are capable of mimicking human NB, we injected NCC obtained from patient-derived iPS cells into the right ***milieu***, the adrenal gland.

Generation of patient-derived iPS cells - Paper I

The NB patients used in this study belong to the same family and carry a germline ALK R1275Q mutation (Figure 15). Skin biopsies were used to establish fibroblast cultures and subsequent generations of iPS cells. To establish patient-derived iPS cells, NB1, NB2, and NB3 patient fibroblast cultures were set up using enzymatic dissociation from skin biopsies as previously described [214]. Unfortunately, due to technical issues during the isolation of fibroblasts, NB3 cells could not be successfully established. Primary patient fibroblast cultures, of NB1 and NB2, were not passaged more than 5 times previous of initiating reprogramming to avoid induction of senescence [215]. Of note, patient fibroblasts were first seeded on inactivated human foreskin fibroblasts, and subsequently maintained in different cell culture conditions. SeV (OSKM) reprogramming was utilized, as it presents a non-integrative and relatively efficient method compared to other non-integrative systems (Figure 8). Importantly here, using a non-integrative method is essential to avoid risk of mutagenesis and implication of other variables during tumorigenesis, assessed in **paper III.**

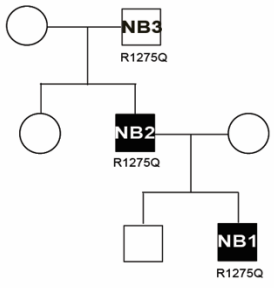

Figure 15. Family Pedigree. NB3 patient was not diagnosed but carried ALK R1275Q mutation. NB2 and NB1 patients were diagnosed with NB and carried same germline mutation in ALK.

After fibroblast were transduced colonies were manually picked around 4 weeks later (Figure 16). Several clones were established from each cell line and frozen down for further analysis.

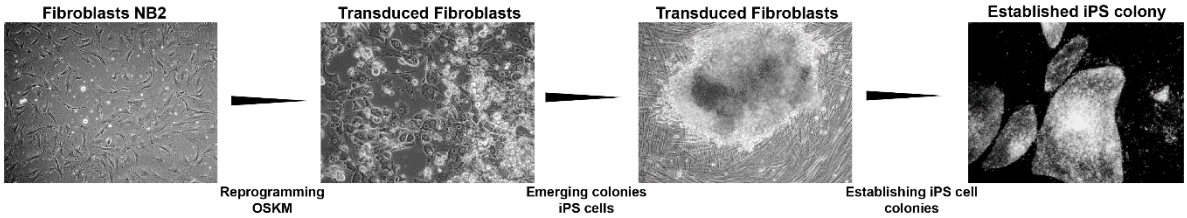

Figure 16. Example of reprogramming flow. NB fibroblast reprogramming in a feeder-dependent system using Sev.

In **Paper I**, we picked one clone of each cell line resembling ES cells morphology, including sharp edges, big nuclei and little differentiation. As previously mentioned, SeV can stay within the cell for several passages. To make sure the iPS cells were endogenously expressing pluripotency factors we passaged them for at least 12 times and confirmed loss of SeV using PCR before proceeding with the validation.

First, iPS cells derived on a feeder-dependent system were validated for pluripotent gene expression using cytoimmunochemistry and the pluriTest bioinformatics tool. Importantly, improvement of cell culture conditions were shown to stabilize pluripotency, increase efficiency of reprogramming, standardize culture of iPS cells and decrease the chances of chromosomal abnormalities [120]. Both are relevant features to establish long-term cell culture of iPS cells for any potential purpose. Since cell culture conditions are important for the maintenance of pluripotent stem cells we decided to optimize our culture conditions based on publications using a defined and xeno-free system of medium in combination with recombinant human laminins [216]. In 2000 Amit et al, identified that bFGF supplementation was required when FBS was removed from the medium composition [217]. Examining this, already in 2006, a defined medium termed TeSR1 was developed for usage in feeder-free conditions. This medium developed by Ludwig et al [123] solely included recombinant sources of human purified proteins; for example high concentrations of FGF-2, lithium chloride, γ-aminobutyric acid (GABA), TGF-β, and pipeolic acid. More recently, TeSR1 was chemically reduced by diminishing all the components to a number of eight (E8), lacking for instance serum albumin and β-mercaptoethanol thereby reducing a source of variability [218]. Because of all the benefits that defined medium combined with feeder-free conditions supported, we decided to transition our iPS cells to defined E8 medium. Concerning the extracellular matrix, matrigel is the most common and widely used matrix to culture iPS cells. However, due to its animal origin and batch-to-batch variability it did not fit the purpose of standardization of cell culture conditions that we aimed for. Laminins are heterotrimeric proteins and components of the ECM containing an α, β, and γ chains that are found in five, four, and three different genetic variants respectively. LN-511 and LN-521 have been shown to be expressed in the human pre-implantational embryo and ES cells. First it was shown that LN-511 could maintain self-renewal of human pluripotent stem cells for more than 20 passages [127, 219]. Later on, long-term self-renewal of human iPS cells was supported by LN-521 as well [216]. Therefore we decided to combine E8 medium with LN-521 in order to obtain defined and xeno-free conditions. After the transition to the new cell culture conditions, we evaluated the expression of pluripotency

markers on our iPS cells. Patient iPS cells expressed NANOG and OCT4 at mRNA and protein levels. Pluripotency markers such as SOX2, SSEA-4, and TRA-1-81 were assessed at protein level either by immunochemistry or flow cytometry. Furthermore, flow cytometry analysis indicated a homogenous cell population of undifferentiated cells which demonstrated the reliability of our conditions. Moreover, SeV backbone was detected in early passages of iPS cells whereas it was no longer present in later passages. This implied that expression of pluripotency markers was indeed endogenous and not promoted by the reprogramming factors. Previously, we mentioned that PluriTest is a bioinformatic tool that facilitates validation of iPS cells via interrogation of big data sets of somatic and pluripotent expression profiles. The generated iPS cells were scored for pluripotency and novelty which are parameters defined by the test. The pluripotency score is based on a pluripotency signature whereas the novelty score indicates how similar the samples of interest are compared to already established and characterized pluripotent stem cells. PluriTest was assessed before and after the change of culture conditions and was shown to not be affected when compared to a feeder-dependent system (data not shown, Kele et al, Manuscript). NB1 and NB2 iPS cells were confirmed to be karyotypically normal and retained the ALK mutation. ***In vitro*** differentiation was assessed using EB formation, after 3 weeks mRNA expression of ecto-, endo-, and mesoderm markers were evaluated either by RT-PCR or qRT-PCR. We observed that markers of the three germ layers such as NCAM, RUNX1 and AFP were upregulated compared to iPS cells. This demonstrated the potential of the obtained cells to differentiate into all the three germ layers. In summary, in **paper I** we show the generation of fully characterized iPS cells from two different NB patients carrying a germline mutation in ALK.

Human neural crest cells from pluripotent stem cells using robust, defined, xeno-free conditions - Paper II

Next, we needed to establish a protocol to derive NCC from the patient-iPS cells. NCC have a great multi differentiation potential and its development occurs during gastrulation dependent on the BMP and WNT signaling pathways [220]. Ethically, studying NCC biology in humans has been limited to just few observational studies in embryos [221]. Therefore, most of our knowledge is based on studies of vertebrates such as zebrafish, xenopus and chicken. It has been established that the mesoderm produces antagonists during neural tube formation, that modulate BMP signaling and help the specification of neural, neural crest, or epidermal lineages [222]. It is clear that BMP activity is important for NCC differentiation but the mechanisms underlying this process are poorly understood. ***In vitro***, BMP ligands are usually present in the media or produced by the cells that makes it overall difficult to dissect out other variables influencing induction [223]. This, and different needs during temporal differentiation, are a few of the reasons why some protocols rely on and other protocols avoid usage of BMP inhibition [165, 166].

In vivo, human NCCs were shown to express markers, like SOX10, PAX3, TFAP2a and p75 neurotrophin receptor [(NTR)], that are also present in other vertebrates. The genetic network of NCC induction, differentiation, and specification is complex and transient [158]. The induction of BMP, FGF, and WNT among pathways during development, triggers expression of specific

transcription factors that can be used as markers. Examples are; border specifier genes Pax3 and Pax7, signaling molecules TFAP2a, SOX9, and SOX10 as neural crest specifiers that are relevant during migration and differentiation of NCC [221]. As mentioned, some of these genes have been studied in other systems indicating their relevance during NCC development. For example, PAX3, SOX10, and TFAP2a mice mutants display NCC defects [224, 225, 226]. *In vivo* human embryo studies demonstrated the presence of PAX3, SOX9, SOX10 in pre-migratory NCCs during the 4th gestational week. Moreover, additional rostral sections indicated the presence of TFAP2a and p75 ^{NTR}.

In **paper II** we demonstrate that controlled intermediate levels of BMP promote NCC differentiation in a robust and efficient manner. The developed protocol is defined and xeno-free method which provide robustness, less variability, and more reproducibility. To establish this, we modified a protocol based on SMAD dual inhibition and WNT activation (NCN2 protocol). After 7 days, we sorted for p75 ^{high} (++) expressing cells which have previously been shown to be enriched for SOX10, PAX3, TFAP2a and concomitantly downregulation of the pluripotency marker OCT4. NCC-derivatives were generated to validate NCC identity. In order to do so, a SOX10-GFP reporter ES cell line was used. Based on GFP expression, SOX10 cells were sorted and differentiated into mesenchymal cells, osteocytes, chondrocytes, as well as peripheral and sensory neurons. Moreover, we found that NCC were highly migratory and expressed ETS-1, a cranial NCC marker. Altogether showing the multipotency and functionality of the generated NCC cells. However, the NCN2 protocol did not always produce NCC with similar efficiency or quality, suggesting that the protocol needed more optimization. We observed that efficiency correlated with endogenous levels of BMP4. In case of too low BMP4 levels, BMP4 was added to the media resulting in increased efficiency of NCC generation. On the contrary, the addition of Noggin, a BMP inhibitor, led to a decrease in the efficiency of NCC generation. Thus, NCC differentiation requires precise levels of BMP4. To avoid fluctuations in BMP4 levels during NCC differentiation a "top-down inhibition" (TDi) system was developed, which involved saturating cultures with exogenous BMP4 while blocking endogenous BMP4 production using a BMP type 1 receptor inhibitor (DMH1) (Figure 17). This resulted in controlled and stable levels of BMP4 throughout the differentiation protocol and permitted the cells to differentiate optimally to NCC and avoid induction of genes that are associated with non-neural ectoderm, meaning that the use of TDi reduces variability and increases efficiency of generation of NCC from human pluripotent stem cells.

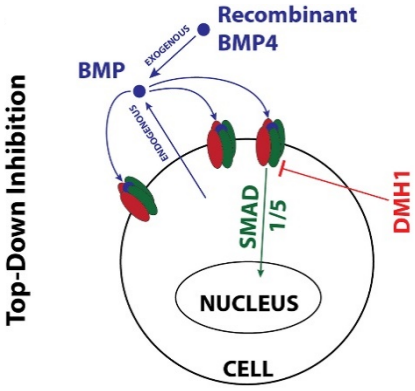

Figure 17. Top down-inhibition strategy. Hackland et al, Stem Cell Reports, 2017, with permission from the publisher.

Modeling NB in vivo – Paper III

In Paper I we demonstrated the generation of patient-derived iPS cells from two NB patients with ALK R1275Q mutation [212] and in Paper II we developed a robust protocol for generating

NCC, the cell type thought to be the origin of NB. In **Paper III** we applied the NCC differentiation protocol on the patient-derived iPS cells to study the contribution of the ALK R1275Q mutation on tumor development. First, we transduced the iPS cell lines with a luciferase reporter in order to monitor them ***in vivo***. Subsequently, we differentiated iPS cells into NCC using the defined and xeno-free conditions established in Paper II. We derived NCC from a fully validated control iPS cell line (Ctrl7) [227], which successfully differentiated into p75 ^{high} positive cells and exhibited the elevated NCC markers PAX3, SOX10 and TFAP2a. Minimal variability in gene expression profile was observed between three separate inductions demonstrating the robustness of the NCC protocol. Next we proceeded with assessing the NCC differentiation potential of the patient-derived iPS cells. Both NB-iPS cell lines were able to generate a p75 ^{high} cell population and NCC markers were observed at mRNA and protein levels. While we could not detect any differences in NCC induction efficiency or marker expression between control and patient derived NCC, we observed an upregulation of MYCN in patient NCC compared to control NCC, indicating a possible collaboration between ALK and MYCN in the patient cells. ***In vitro*** studies in patient NCC need to be further evaluated in order to identify possible differences of NCC and derivatives between patient and control NCC.

In order to establish a NB model from patient-derived NCC, we sorted p75 ^{high} cells from control and patient cells. We proceeded to surgically inject NCC orthotopically into the adrenal gland of 6 to 8 weeks NOD/SCID/IL2R$\gamma^{-/-}$ (NSG) mice. IVIS imaging was used in order to follow progression of growth ***in vivo***. While mice injected with control cells did not show any symptoms for a period of almost a year, the luciferase signal increased in some of the patient-derived NCC after 24 weeks post-injections. After one year, the adrenal glands were harvested for immunohistochemistry analysis. H&E staining and different NB diagnostic markers such as PHOX2B, and NCAM, identified that in a cohort of 12 mice, one NB1-NCC injected mice developed ganglioneuroblastoma. Ganglioneuroblastoma is a more benign and differentiated form of NB (NB1NCC#3) [228]. On the other hand, mice injected with NB2-NCC, that presented a slower increase of the luciferase signal than NB1, developed an undifferentiated structure which was unable to identify utilizing available NB diagnostic markers (NB2NCC#12). Moreover, we observed calcifications and bone formation upon injections of patient-NCC (NB1NCC#9) indicating the potential of NCC differentiation into their derivatives ***in vivo***. Indeed, the diversity of our results demonstrates that the mice cohort needs to be increased, in order to confirm low penetrance of ALK driven-NB. However, it would be interesting to analyze the ALK activation pathway during NCC derivation and differentiation, to understand when ALK is active during this process and whether tumorigenesis is originating due to this driver mutation.

Overall, we present a new way of utilizing patient-derived iPS cells to model NB which has the potential to reveal ALK implications during NB initiation and progression.

3.2 MODELING SHH-MB USING IPS DERIVED NES CELLS (PAPER IV)

Medulloblastoma (MB) is the most common childhood brain tumor and develops from a progenitor population in the developing cerebellum.

Human neuroepithelial stem cells (NES) are neural stem cells residing in the neuroepithelium during brain development. NES cells have the ability to self-renew and also differentiate into RG or neurons taking an important role during neurogenesis. It is possible to capture NES cells from early embryos but they can be derived from iPS cells as well [154, 229]. iPS derived-NES cells show a gene expression profile closely resembling human embryonic hindbrain identity. Therefore, NES cells match the cerebellum position identity and thus present an ideal tool to model MB. ***In vitro***, long-term NES cells are capable of self-renew for more than 100 passages when cultured in FGF and EGF conditions and 90% differentiate into neurons while 10% differentiate into glia upon removal of growth factors. Overall, the position identity and differentiation behavior demonstrate that NES cells are an important tool to model MB [229].

Gorlin syndrome (also named Nevoid basal cell carcinoma syndrome) patients are prone to develop basal cell carcinoma, as well as MB, due to a germ line mutation in one allele of PTCH1 and resulting in aberrant activation of the SHH-pathway. In Paper IV we aimed to model SHH-subgroup MB. In order to model SHH-subgroup MB we used non-cancerous cells from two Gorlin syndrome patients carrying different PTCH1 germline mutations, which we refer to as G1 and G2 respectively. Both PTCH1 mutations lead to frameshifts resulting in PTCH1 protein truncation. Keratinocytes of these patients were reprogrammed into iPS cells and differentiated into NES cells. When grown ***in vitro***, we found that Gorlin NES cells did not show differences compared to control NES unless grown in a 3D system or in hypoxic conditions. The lack of difference suggests that SHH pathway activity might be lost in cells grown in a monolayer [230]. Next, Gorlin NES cells were transduced with a luciferase reporter and were orthotopically transplanted into the cerebellum of immunodeficient mice and followed ***in vivo*** using a bioluminescence ***in vivo*** imaging system (IVIS). Luciferase signals were detected in mice from 8 weeks post-injection with Gorlin patient cells, whereas no signal was detected up to 52 weeks post-injections with control cells. Upon harvasting and subsequent investigation of mice brains we observed tumors in the cerebellum. The tumors consisted of small, round, and blue cells and were either of desmoplastic or classic MB histology which resembles human SHH MB samples. The tumor cells could be isolated and cultured ***in vitro*** as neural stem cells (referred to as tNES from here) and generated secondary tumors with accelerated tumor growth when re-injected into mouse cerebellum.

Tumor cell gene expression was analyzed by RNA sequencing (RNA seq), and using principal component analysis (PCA) was found to group with human MB tumors Furthermore, profiling using expression data from human MB samples revealed that the tumor cells, primary and secondary tNES, showed a gene expression profile resembling SHH-subtype of human MB, demonstrating that Gorlin patient NES cells form tumors ***in vivo*** that faithfully mimic human SHH-driven MB.

Gene set enrichment analysis generated from tNES RNA seq, revealed a progressive upregulation of inflammatory and EMT pathways in the tumor cells. Interestingly, we found upregulation of LGALS1 or Galectin-1 which has been correlated with metastasis and poor prognosis in many types of cancer. Especially in the SHH-subtype of MB, high LGALS1 expression is correlated to worse overall survival. Thereafter, to investigate the importance of LGALS1 we used OTX008, a specific Galectin-1 inhibitor, we used OTX008, a specific Galectin-1 inhibitor. Treatment with OTX008 led to decreased proliferation of Gorlin but not control NES cells, suggesting LGALS1 as a new potential target to treat SHH-driven MB.

To summarize, in paper IV, we demonstrate that NES cells generated from reprogrammed non-cancerous somatic cells carrying a PTCH1 germ line mutation give rise to MB when injected into immunocompetent mouse cerebellum. We created a novel human cell model of SHH MB using NES cells generated from Gorlin iPS cells, and studied their tumorigenic potential ***in vivo***. We believe that our model will lead to the discovery of novel oncogenic mutations, which in turn help us to better understand MB initiation and progression. Thereby, this approach may prove beneficial for drug screening purposes.

3.3 P53 IN HUMAN BRAIN DEVELOPMENT (PAPER V)

TP53 is a tumor suppressor gene that is mutated or inactivated in 50% of all human cancers. Acting as the guardian of the genome, it regulates many processes in the cell including, genomic stability, cell cycle arrest, and differentiation. These functions are extensively studied in a cancer context and to some extend in mouse neural stem cells. In paper V, we use a 2D and 3D ***in vitro*** approach to assess the role of p53 during human neural development.

3D cerebral organoids derived from iPS cells have been shown to closely mimic early brain development both transcriptionally and epigenetically [186, 231]. To further investigate the role of p53 in neural stem cell function and differentiation, we used a 2D monolayer of human NES cells. We believe that the combination of both ***in vitro*** systems can aid understanding of specific functions of p53 during brain development. Figure 18 represents both the complexity and homogeneity of different systems. Whereas 2D ***in vitro*** systems such as NES cells are highly homogenous, 3D whole brain organoids resemble complexity of the human brain more closely. Current 2D models, although versatile and homogenous, quite often contain pure cell populations with solely one cell identity. Despite its limitations, the potential of this system is immense, especially considering a certain lineage specification (See example in paper IV). On the other hand, due to the formation of different types of cells and stem cell niches such as the VZ, SVZ, and neuronal layers 3D systems offer a higher complexity. However, spontaneous self-organization and heterogeneity usually result in increased variability. Nevertheless, 3D systems present a good tool to investigate pathogenesis underlying strong phenotypes as has been shown previously [168]. .

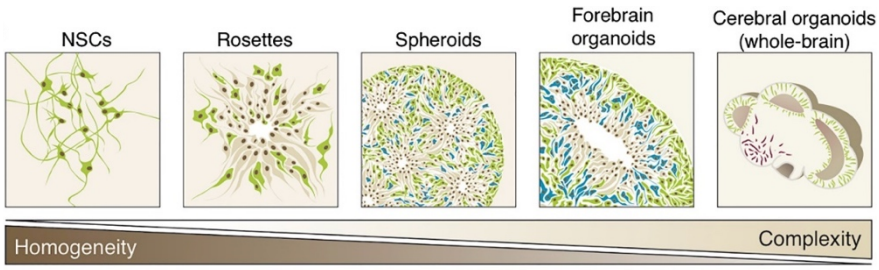

Figure 18. Homogeneity and Complexity of neural ***in vitro*** systems. Kelava et al, with permission from the publisher, Cell Stem Cell.

In **Paper V**, we used two different iPS cell lines derived from healthy individuals [232] and three different lentiviral shRNAs, two shRNAs target different regions of TP53 and one is a control shRNA. We knocked down (KD) p53 and generated whole brain organoids. We observed that p53 KD organoids are able to form self-organizing structures, albeit with less efficiency in generating cortical tissue. For these reasons, we evaluated different stages of the developing cortex. Whereas SOX2 expression is a good indicator of the NSC layer in the brain [233] [234], TBR2 and TBR1 positive cells suggest NSC differentiation towards post-mitotic projection neurons [235]. Sequentially, TBR2 is expressed in intermediate progenitors and usually emerges after downregulation of PAX6. Whereas, TBR2 is downregulated in later stages of cortex development TBR1 expression is present. Therefore, to gain a general overview of cortex development, we analyzed SOX2 expression and quantified the key cortical developmental markers TBR2 and TBR1 in whole-brain organoids. We observed delocalization of the stem cell layer recognized by expression of SOX2. Quantification of TBR2 and TBR1 revealed a reduction of TBR1+ neurons generated in the p53 KD cortex. Since this indicates that either the stem cell function or differentiation potential of the NSC were impaired, we proceeded by knocking down p53 in a 2D ***in vitro*** system of NES cells. Upon loss of p53, NES cells still exhibit stem cell markers and classical rosette-like morphology. This suggests that loss of p53 did not affect self-renewal properties. Furthermore, analysis of the cell cycle revealed that cells accumulate in the G2/M phase. In combination with detectable amplification of centrosomes, it pinpoints p53's role in maintaining cell cycle progression and centrosome regulation in human neural stem cells. To gain a deeper understanding of the functions that p53 plays in human NSC we analyzed the transcriptome of p53 KD NES cells and found that metabolic pathways such as oxidative phosphorylation were downregulated. Among the upregulated processes, we observed CNS neuron differentiation. Since the NES cells were still expressing NSC markers, but transcriptomic analysis indicated a priming towards differentiation, we differentiated the NES cells into early neurons by removing growth factors in the media. Functional analysis of these neurons revealed a more mature state after 15 days than control neurons, as indicated by an increase in calcium oscillations. Furthermore, after 30 days of differentiation, the p53 KD neurons gained oxidative phosphorylation as a main source of energy, indicative for maturing neurons and correlating with previous calcium data.

In conclusion, we demonstrate that p53 is required for the proper localization of neural stem cells in 3D organoids. In addition, by using a 2D NES cell system we unraveled the involvement of p53 in genome stability and energy metabolism of human neural stem cells. Whereas 3D brain organoids mimic the ***in vivo*** situation of neocortex development, including structural and cellular complexity, NES cells are a much more homogenous system with hindbrain identity. The 3D organoid system offers the investigation of p53's role in brain development, starting from p53 downregulation in the iPS cell, before brain formation. At the NES cells stage we captured occurring processes during a specific timeframe and did not consider previous events before generation of NES cells. We believe that by using a combination of tools we can examine the role of p53 from different perspectives and in a continuous manner throughout brain development. Using p53 KD brain organoids we clearly observed abnormalities in the structural organization of the stem cell layer and lower efficiency of generated tubular regions, including significant downregulation of the cortical marker TBR1. However, possible functional experiments are more complicated to assess in this system. 30 days old brain organoids, which is the time when the neuroepithelium establishes, were examined. This time point has previously been shown to resemble closely the human brain development (Figure 13). Therefore, after studying organoids exhibiting delocalization of the stem cell layer upon loss of p53, we moved on to the NES cells system to further examine the role of p53 in a more simplistic manner. For example, the NES cells system allowed for the detection of several phenotypes which were intricate to uncover in the brain organoid system. Furthermore, investigating the number of centrosomes per cell and to functionally explore the differentiation potential of human NSC was only able in the NES cell system.

We believe that these results aid to the understanding of p53 in human brain development and function in an otherwise mouse model and cancer research dominated field.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

Cancer is one of the most common leading causes of death worldwide. Even though childhood malignancies are rare, they still are the most frequent cause of death in children after infectious diseases. Current therapeutic options to treat children can have severe permanent side effects. Childhood cancers occur during early human development which implicates difficulties in treatment possibilities as well as limitations in finding the origin of the disease. The main aim of this thesis has been to establish new ways of modeling childhood malignancies to facilitate a future understanding of the onset of the disease as well as a new potential tool of anti-cancer drug identification.

In order to do so, we took advantage of the immature state of iPS cells which have been derived from NB and MB patients. Next, we successfully derived different ***in vitro*** progenitors such as NCC and NES cells which belong to specific developmental lineages during PNS and CNS development. We wanted to investigate the effects of non-cancerous cells carrying germline mutations that predispose to cancer when injected into a permissive environment. After generating all the resources needed we used immunocompromised mice to inject the respective labeled progenitors into the right environment and monitored them in ***vivo***.

NES cells are a robust, well established ***in vitro*** system with hindbrain identity, representing a perfect tool to mimic MB. On the other hand, NCC are much more transient and unfortunately, we were not able to capture them ***in vitro*** for an extended time. Whereas the data shown for the NB model still is preliminary, the MB model allowed us to developmentally mimic the initiation of human MB, establish ***in vitro*** NES cultures from MB tumors from mouse cerebellum, and use the cells for the identification of potential clinical targets.

This work presents the possibility of studying many inherited mutations in cancer or associated syndromes. The iPS cell technology opens new possibilities and approaches to study complex human diseases. It proves especially advantageous for those with early onset. We believe that understanding the initiation and development of cancer from the first hit will aid identifying potential new clinical targets, improve the current treatments, allow a better and more accurate prognosis, and to possibly find new biomarkers.

On the other hand, brain organoids derived from iPS cells have been a big revolutionary tool to study the developing human brain. The work presented here merges different human ***in vitro*** systems to understand the overall function of p53 during human brain development. Of note, to our knowledge all previous reported studies have been performed in mice. p53 is an important player during cancer development, however mutations in p53 mostly occur in adult cancer and during later stages of the disease. Cancer is a heterogeneous disease which does not solely depend on cancer cells, but surrounding tissue and associated microenvironment are key components for the initiation, progression, and spread of the disease. More recently, tumor organoids have been generated from many primary cancer tissues and have been shown to be useful in identifying potential therapeutic approaches [236]. Moreover, an organoid biobank from patient-derived healthy and tumor tissue samples has been established which brings us closer to personalized cancer treatment [237]. These are only

a few examples that indicate the big potential of the organoid field in cancer research. We believe that iPS cells derived organoids could be utilized not only to understand development but also to understand the initiation of many cancers using the strategy that we present in this thesis.

Summary

(i) To develop *in vitro* and *in vivo* models using patient-derived iPS cells to mimic early developmental malignancies; Neuroblastoma (NB) and Medulloblastoma (MB).

Paper I

- We successfully establish and characterized iPS cells from two NB patients with familial ALKR1275Q mutation.

Paper II

- We generated a fully defined and xeno-free protocol for generation of human NCC from pluripotent stem cells.
- We could identify endougenous BMP production as a source of variability for NCC differentiation *in vitro*, and by controlling the level of BMP4 during NCC differentiation we could increase the reproducibility of our NCC protocol.

Paper III

- We showed that NB patient-derived iPS cells are able to differentiate into NCC.
- Orthotopically injected NB-patient NNC are able to survive *in vivo*, thus making it a potential new model to study the contribution of ALK mutation in NB development.

Paper IV

- We faithfully model human SHH-MB using Gorlin patient derived-iPS cells.
- We showed that tNES are a potential model to use for identification of potential novel targets for MB.

(ii) To study the role of p53 during human brain development.

Paper V

- We demonstrate that p53 is required for neural stem cell positioning in whole brain organoids.
- We show that loss of p53 leads to genomic instability and changes in metabolic energy requirements in human neural stem cells
- Upon p53 lost we show that NES cells are capable to differentiate into neurons reaching maturity faster than control NES cells.

5 ACKNOWLEDGEMENTS

A big thanks to **EVERYONE** that have supported, encouraged and make me believe in myself until the very end of this journey; to my dearest family, friends and colleagues. All of you are a huge part of my life, and without you this definitely would have not been possible! There are no words to describe how grateful I am to have met you and shared with you the most memorable moments during this period.

First, I would like to thank my main-supervisor, **Margareta Wilhelm**. Thank you Maggan for have given me the unique opportunity to grow as a scientist with very strong ethical and moral values. As a role model you are for many of us, it's your sharpness, creativity, good science and kind soul that have always inspirited all of us. It has been a long journey with many challenges that together I believe we have managed to overcome. Thanks for your immense support, consistent and almost blind trust in me. Thanks for helping me to reach my dreams, for including me in your lab, for making me feel part of your life and take constant care. Thanks for teaching and mentoring me, for giving me the best input, for always having an answer to my questions. I am so proud of everything we build up together and I just hope it is something will persist and be helpful for your lab and your successful future. It was a big pleasure to work with you during the past years. Simply thanks because without you, I would not have made it.

Thanks to my co-supervisor **Anna Falk** for accepting half of the deal and therefore always including me in the "neuro" lab. Really thanks for being so flexible and allow me to work in the iPS core for all this time, which fulfilled many of my dreams working daily with iPS cells!.

Thanks to my co-supervisor **Per Kogner** that even though we haven't had the opportunity to meet that often, you gave me the chance to develop "my baby" project. I still remember the day you signed my admission forms and said to me: "Ana, I hope you understand the risk of this project". Believe me, even though I positively nodded at that time, I did not know, but I know now ;).

Thank you **Marie Arsenian Henriksson**, that even though officially you are not my supervisor, co-supervisor or mentor…I need to immensely thank you for your big support throughout the years. For your generous words, giving me the strength to keep going. For noticing when something was wrong, when none could. For always finding a way to help. I admire your strength and values Marie, you do such excellent job and I wish the best future for you and your lab as well.

I would like to thank my two mentors; **Fredrik Lanner** for always being available if needed and for being such a good role model in science, I hope the best for your future and your lab!. To **Jesper Ericsson** for inspiriting me so much with your passion and motivation. Thanks for our lunches and inspirational conversations, they have meant a lot.

For the past years, I have worked physically in two different labs and shared space with another. This has given me the incredible opportunity to meet and share experiences with exceptional people during the past years.

To **Robin** or Robbit, depends on who you ask XD… I just simply have no words to describe how grateful I am to have met you and to have a friend like you. But I'm going to try. You are the kindest and most caring person I have ever met. "I don't care, I'm dutch", does not work for me because you mean right the opposite. I am so so so thankful to have you in my life and I am so so so happy about it that I would actually need an entire book to write down the million things we have done and shared together, inside and outside the lab; from days and nights in the lab to the best nights out to discovering so many different countries together (and the hundred million other things that are still left to do!). I have LOVED working with you, side by side, endless days and nights. I would have never managed half of it without your support, persistence, friendship and love for science. I will miss it a lot, but the bench would have NEVER been as fun without you. Thanks for teaching me so much about literally everything, for caring, for ALWAYS ALWAYS ALWAYS being there no matter what, how or when. You are undoubtedly my biggest pillar during the past years, and this thesis would not have happen without you. YOU WILL DO SO GREAT, NEVER DOUBT IT. THANK YOU!!!!!! I love you **<3.**

Thank you **G <3**.I am so fortunate to have had the opportunity to share the lab with someone like you. I got to know one of the most awesome people I have in my life ☺. You have always made me feel so protected, and so taken care of. And for sure, you will recognize this sentence but, you have always been there when I needed someone to hold me up. Thanks for being such a good friend, for sharing and being so understanding, for your ENDLESS support and help during literally all these years. For guiding me through this with all your incredible wisdom. For always finding the words to cheer me up, for being there, from the very first day to the very last in the lab!. Thanks for ALL the help in everything. Yes, also including the seahorse experiments, that counts as well XD. I love you G.

Thanks to the rest of **Maggan's lab** members, past and present. To **Evelyn,** cheers to our rough beginning and amazing development towards a very fruitful friendship. I am very happy we could meet each other as we are and work together like a team, especially in the most difficult times. Thank you for teaching me and trusting me. I wish you the best luck in your new career path, you will blast it!. To **Jojo,** thank you for your kindness and understanding. Thank you for always trying to figure how to release me from tasks that were stressing me out, I always have appreciated a lot. Thanks for caring and for joining Squash!. I see you in the court ;). Thank you **Habib** for always be willing to listen, talk and discuss about life. I am so happy for you and your new position and I hope the very best in your future. To **Marina,** thank you for your generosity and willingness to help in everything that has been possible! You know it has been intense for me and you always tried to mild it down. Thank you. **Aitor,** muchísima suerte con todo! **Okan,** thanks for all the funny conversations. Best luck in your next step!

Thank you to everyone in **Marie's Lab,** also past and present. To **Johanna** for teaching me everything I know about Squash ☺. For your truly kindness and being such a good friend. Even though you are in the distance now, I don't forget you!!. **Diogo!** You are such a source of inspiration! A massive thank you for ALL your coaching and guidance. For all the help inside and outside the lab, for all the fun times!. To **Mariavi** and **Lourdes,** I think you guys deserve to have the names close to each other haha. Mariavi you are such a good person and scientist. I admire you so much and I genuinely wish you the best in your future. Lourdes, you make me laugh so much! I am so happy you joined Marie´s lab to get to know you. Sabemos que hay cosas que solo las dos podemos entender ;). I am certain you will do amazing in your PhD!. **Aida**, thanks for helping, understanding and always caring about others. I wish you the best in your future!. Thank you **Elena** for our small talks regarding our home town, you always make me smile with that! Thanks also for always caring. Super good luck with your next step! **Tong,** sorry for always be in your way every single time that we run a WB. I am really unsure how I manage but probably you have noticed lately that you don't fear anymore of dropping membranes and buffers! Good luck with everything!. Hej Hej **Oscar** ;), I thought I would include you here haha!. At the end of the day… the lab is how we got to know each other. Thank you my friend for all the extremely fun times, in Stockholm and Gothenburg! Thanks for your infinite and unexpected caring calls, they always make my day. I wish you the best luck in your medical studies!.

Thanks to all the members of the "**neuro lab**" and **iPS core** past and present. Thank you immensely to **Harriet** and **Kelly** for always allowing me to work, share and teach in the core facility, THANK YOU for make one of my dreams come true. **Harriet,** I have so much to thank you… From always welcoming me to the core, to hosting us during Christmas parties in your place, nights over your cabin, saunas, wine, glöggs, laughs and conversations…I admire your patience, understanding and wisdom. You are such a strong and admirable woman!. To **Kelly,** the sweetest, kindest and sharpest person ever. I don't know how to thank you for always listening and giving me the best advice. Thank you for your immense patience and tranquillity. Thank you for the past years in the lab, allowing me to learn and develop. Thank you for your truly friendship and for literally saving Robin and I in many occasions. You are so so good!!! And I have no wonder how brilliant your future will be. Thank you also to **Mastoureh**, your love and warmth has no limits. I really need to thank you for every time that you were there to cheer me up with your words. I will always appreciate how much you care and last but not least, how funny you are!! I wish you the best to you and your family ☺. **Mohsen** thank you for all the fun and priceless long island ice teas, mojitos, beers, wines and vermuts!! Thank you for caring and taking care of everyone. Thank you **Salma**, for always taking care so much of cell culture and being so sharing. Thank you **Malin** for helping in everything your time allows. Good luck with everything!. **Elias**, **Matti**, you are next in the queue, good luck!. Thank you **Philipp** for your amazing aura and hang outs!.**Rebequita**, disfruta mil!

A big thanks to **Sonia Lain's group** which I spent a year before starting my Master's degree at KI but a life changing experience. Muchas gracias **Sonia** to give me the opportunity to do my exchange in your lab, you literally open the doors for me to develop as a scientist and follow my gut feeling. **Anna**, I will never forget you, you were my very good friend. You always supported me and took care of me in the lab. Your lost was very painful but you have been always with me. I know I made a promise to you that I would keep strong during my PhD. Here I am today, it is almost done and I feel you have been always close to me for the entire journey ☺. Gracias **Inge** por todo lo enseñado. Tú me entrenaste para trabajar duro y ser consistente. Tu pasión y coraje siempre me han admirado, muchísimas gracias por formarme!. **Marcus**, thank you so much for your comprehension, funny stories and especially, teaching me bad words XD. Thank you for helping me with all my questions before and during my PhD!. You will do amazingly good during your Postdoc!. Thank you **Gergana** for helping me so much with the migration assay experiments, without you that experiment would have never happened. I appreciate a lot your efforts and willingness to help me. Thank you! And **Andrés** por ser la alegría de la huerta! Muchísima suerte con tu futuro (quien sabe quizás mañana estás de vuelta!). Thank you **Marijke** for always teaching me in the lab when was needed and for your guidance during the past years.

A big thanks to all the rest of **MTCers**, because you guys rock! You made these years full of laughs and extremely fun times!. **Amandaaaa**, separated by one simple floor for so long…it took us a while to get know each other ^^, you are my other half and you know in which skill we both excel, I don't have to mention it!. Thank you because I have always a blast with you, with or without the tequila! XD <3. Thanks **Jonathan** for all the support and knowledge, and beers (that last one is also important). You are such an inspiration! Keep it always so cool! **Joanna and Miland**, you will do amazing in the US and I hope to see you guys really really soon! Thanks for all the fun times in the MTC pubs. **Jacob,** I miss you over here! It was such a great pleasure to share those 2 years at MTC, thanks for the good times and friendship. Sorry I got so sick in Australia :D. I will pay that one back!. **Wisam** was nice to share same floor and share small talks to realise some of our pressure. Thank you **Marc** for the super fun times before you left to US, it is great to have you back!. Good luck!. Thank you **Birgitta**, you literally saved me with your live sorting FACS skills when the biggest experiment I ever run was on going and almost about to fail!. I owe you a year experiment! Thank you for all your teaching and your love for these machines, I definitely inherited it!. Thank you **Kenth** for taking care of my little "mousies". They meant a lot and you always made sure everything was in place!. **Adam,** I know you weren't MTC but for me like you were, since anyhow the animal house has been always our meeting point. Thank you so much for all the support, massive help with the IVIS (you thought me everything I know) and your trust. You are simply a star! I am so happy for your new position!. Thank you **Torun** for being so great in the animal house, working with you made totally a difference. Thank you **Per** for always fixing my laptop

as soon as you could. You know by now much of a disaster I am. Thanks to **Åsa** and **Gesan**, for all your support and absolute dedication for PhD students wellness and success.

Thanks to **all collaborators** that made this work happen ☺. A big thank you to **Peter Andrew's lab.** Thank you **Peter** for giving me the opportunity to join your lab for a while. It was an amazing personal and professional experience and an absolute key contributor for the success of our work. Thank you so much **Christian** for fixing my stay in the lab and taking care of me during that time. And a massive thanks to **Jim!** Thanks for sharing your impressive knowledge and teach me how to generate NCC, it was such an honour to get to know someone that sharp and kind person like you. Good luck in the next step, you will rock it! **Tom**, bro! I can't express how happy I am to have met you, you make me laugh sooo much (you know that). Thanks for all the help in the lab, and even after I left, for answering all of my annoying questions. Thank you so much for taking care and including me in your life during that time. **Both of you,** made that experience irreplaceable, really valuable and extremely positive! THANK YOU NCC TEAM! <3. Thanks to **Daniel Bexell** for believing in our project and kindly teach me one of the most challenging parts of my PhD; Orthotopic injections into the adrenal gland! Thanks a lot Daniel, I really enjoyed doing something so relevant. Thank you to **Miklos**, for taking your time examining all our sections and giving the best of your knowledge.

Thanks to my **beloved students**, for your impressive hard work that always made me feel so proud of you. **Haizea,** gracias por todo! Te echo de menos! Te veo pronto ☺. **Jonne**, thanks for your persistent, good and successful work, I am sorry not all of that is included in this thesis but don't doubt that your work is a key factor for the progression of our work!. **Astrid,** you are such a big part of this. You are a hardworking and so intelligent angel, you literally helped me with everything and on top of that, I learnt so much from you. I am SO happy you came to us. You will do always amazing Astrid! "*The team will never give up" <3.*

Thanks to all my **dear KI friends,** you know that without you I would not be the same and I would definitely have never achieved something like this…Thank you **Elin!** My beautiful Swedish girl <3. Thank you for all these years of true friendship, understanding and especially for all the love you have given me. I love you so much! The journey has been so much smoother having someone like you by myside. For more years to go! *Manzanas* and *Lycka*, are important concepts we have learnt through these years XD. Panda **David,** I am unsure how big of a fuss I can make here…but I just want to let you know that your kind and sweet soul, dedication and good spirit makes you unique. If I can be proud today that my thesis is at the minimum readable, is because of your massive efforts correcting, scratching and proof reading every single sentence on it. I can't be more thankful for what you have done. Thanks for your extreme positive attitude and for being such a good friend, I am so lucky to have you around!. Thanks **Alex** for your valuable friendship and amazing times together! Thank you immensely for your constant caring. Your hard work will pay off and soon, you are also almost

done! I know you will do great!<3. **Eliane,** estoy tan agradecida de haber-te conocido. Eres totalmente la luz que me faltaba. Tan dulce y siempre tan atenta y ciudadora. Muchísimas gracias por cuidar de todos y de siempre estar ahí! Te aprecio un montón. **Michael**, thank you deeply for all the conversation and advice you have given to me, I really appreciate it! Keep putting my feet on the ground!. Thanks **Mat** for all the fun times but especially thanks for running 30 freaking Km together!. I would have not done it without you! I wish you the best luck in your new position, you will do GREAT!. **Daniëlle,** it was such pleasure to include another dutchie in my life! Thank you for the fun times and sharing experiences, hopefully more to come!. **Konstantinos and Agnes,** we need to go back to the training together! and then we will see results XD! so maybe also thanks to **Mark** for the hard core crosstraining classes, it helped me surviving this journey!. **Konsta**, it is unbelievable we know each other since the masters….time just flies. I wish you the best luck in your PhD and I will be there when it is your turn! **Agnes,** you are so sweet! And you are really the next! You can do this!! And with **Vilma,** you guys make the week much easier after meeting every Wednesday! Bagpippers it is! ☺. Thanks Vilma for all your interest and sharing knowledge. Thank you, **Jens**, for your positive mood, always ready for a blast! It was amazing to share the experience in Cold Spring Harbor course, NYC and Barcelona multiple times!. **Diana,** me alegro mucho de haber-te conocido, gracias por encontrar tiempo en Madrid y por todas las conversaciones que siempre he disfrutado mucho. Muchísima suerte, estoy segura que todo irá bien!. Gracias **Sandra** por ser tan rechill, gracias por estar ahí en todos los pubs aunque tuvieras que viajar desde Mordor!. Thank you **Mathew** for all the fun times since your day 0 at KI!. I wish you the best during your postdoc! But let's celebrate 30s first ;). **Christina** thanks for the fun time!

Thanks to all of you **KICC** people! Especially **Giuseppe, Carmen, Theresa and Milos.** It has been a successful half a year thanks to you guys to make all of this happen! Thank you for your organization skills and for being so much fun in all the pubs and parties we have organized. Thanks to all **organizers of the Winter Conference 2019** for your hard work to make it happen! Congrats!.Thanks to the **Roslagshallen Squash club** for all these years of fun and treating me so well.

Thank you to my precious friends from the **Master program!** Thank you **Axel** for being such a good friend, for saving me of living on the street, for always including me in all your big plans like NYE and the best midsommar experiences I ever had! Thank you so much! You are next!. Thank you **Sofie** for your hospitality, kindness, and the best girl nights out ever! Hopefully for more of those to come!. Thanks **William** for all the crazy and amazing times during the masters and PhD, it has been such a pleasure to enjoy all of this with you guys! **Álvarito**, que sepas que siempre te admirado muchísimo! Eres una preciosidad de persona, trabajadora, encantadora e increíblemente inteligente. Te debo los mejores momentos

durante el máster y todas las conversaciones durante los momentos más duros durante este doctorado. Te quiero!

Thank you **Wale** for all the support and taking care of me for the past years! You are such a good friend, I appreciate immensely your efforts trying to understand what is this science related problems about. Thanks **Joel** for your big support throughout the years. For always listening and understanding. Your critical mind has always been extremely helpful, thanks for all the advice!. You are almost done too!

Marina Mandarina, que puedo decir? Indudablemente, conocernos ha sido una de las mejores cosas que me ha pasado en Suecia. Esos dos años viviendo juntas fueron una de mis mejores experiencias de mi vida y definitivamente, no hubiera sobrevivido sin ti. Aún así, todavía me debes un bulle en nuestro muelle ☺. Sabes que te quiero igual que a una hermana y que soy muy feliz de tenerte, pero más lejos de lo que querría :D. Gracias por tu apoyo incondicional durante todo este tiempo y por siempre estar ahí sin importar ninguna distancia. Te quiero mucho!

Gracies a tots els amics de casa. **Mariona, Maria i Amat** són taaaaaants anys (25?). Es increïble! El temps no passa ni passarà per nosaltres. Mil gràcies per tot el suport durant aquests anys. Sé que no és fàcil mantenir relacions a distància i sincerament, vosaltres mai m'heu fallat. Us aprecio i us estimo moltíssim. **Berni,** que puedo decir! Eres el mejor joer! Gracias por estar ahí siempre, por nunca olvidarte de mi en la distancia, por visitarme el que más y por la de risas, aventuras y momentos de felicidad juntos! ¡GRACIAS por la mejor tapa del mundo!

Gracies als trapeTlles de la uni! **Boni i Dani** merci per ser com els meus germans. Per el bon rollo que es veure-us com si el temps mai passés, per tots els momentazos que hem tingut junts. Merci per perdurar a la distància, us desitjo el millor!. **Martí** estic segura que si no fos per totes les hores que vam passar junts estudiant o comentant, mai hagués arribat on estic avui. Merci per tots els grans moments!

A big thanks to the best **Erasmus friends** that are all spread around the world; **Almu,** sin duda una de las mejores experiencias de mi vida fue lo que vivimos juntas durante ese año. Nunca lo olvidaré y aún sonrío en pensar la de tonterías que pasaron. Gracias por todos estos años de soporte y por esta amistad que nunca se ve afectada por ninguna distancia. **Auro and Paul,** such beautiful love story right there! I am so glad to have been witness of that beginning. Thank you so much for your hospitality, it has been amazing to have you over and visit and experience your life in Ghent as well. I wish you could be there for the defence but we will arrange something instead! Auro, ya queda menos! Lo lograrás!. **Karsten** thanks for the eternal support and your big calmness. My favourite Australian, **Nat.** It was AMAZING to see you again after so long. I am so sorry that I got so sick XD. I hope you forgive me for that. I won't forget your hospitality and generosity. I wish you the best for your future and I see you next in Europe!. **Jeff,** I cannot express with words your generosity, you made my trip

to Singapore and absolute amazing experience, I loved it incredibly much and I believe you made it like this. Thank you so much for everything and I hope next time we see each other is not in 6 years from now. **Calum,** I know there would be really few things that would impede you to be here to celebrate, but maybe being thousands km away and have an extremely difficult job like yours is excuse enough ;). You know how much I appreciate our friendship, how much you have helped me going through all the tough moments in the distance. Thank you for always caring and being there "online" when I most needed someone. Thanks for all the visits in Sheffield and Stockholm! Maybe next is Scotland then. Please, take care of yourself!

Thanks to **STEMCELL** people!! Thank you **Andrew** or wait…thanks STEMCELL for randomly choosing me for a travel grant...XD. Ok Joking, thanks Andrew for being such an authentic inspiration. We met at the very last stretch of my PhD, but you have helped me keeping motivated until the very end!. Definitely crucial. Thank you so much for all the advice and enthusiasm!. Thank you **Julia**, for being such an inspiration; your energy, drive and hard work makes me feel so positive. THANK YOU for giving me the opportunity to be part of such an amazing team like yours!. I will do my best! I promise!. **Éamon** Ha! We will rock this! Thank you for being so genuinely nice and extremely helpful! I couldn't ask for a better team leader!.

A mi querida **familia Marín Navarro**, el más merecido de todos los gracias desde el fondo de mi corazón. A mi padres, **Asun y Vicente**, y a mi hermano **Albert.** Gracias por el apoyo sin fin, por inculcarme los valores más importantes de la vida, el trabajo duro, la buena moral y ética, la generosidad y el amor. Gracias por dejarme volar hacia lo más alto, por siempre apoyarme en las decisiones más críticas. Sin duda alguna, sin vosotros nunca hubiera llegado donde estoy. Gracias por todos vuestros esfuerzos, os quiero. Otro gracias para Albert por la foto que has creado para que la portada de esta tesis sea lo mejor de lo mejor! <3. Gracias a **Roser,** por ser la mejor cuñada del mundo!! Gracias por todas las increíbles comidas, cenas, fiestas, risas y mucho mucho amor durante los últimos años. Gracias a todos **mis abuelos, Encarna, Carmen, Juan y Vicente,** los que están y ya no están por formar parte de mi vida, por siempre preocuparse tanto en lo que hago tan tan tan lejos y donde hace tanto tanto frio!. A mi tía padrina, **Carmen,** por demostrar todo tu apoyo incondicional durante tantos años. Por enseñarme a pintar y dibujar y disfrutar de ello desde pequeñita. Gracias a mis **tías, Encarnita y Toñi,** por siempre estar ahí, cuidarme y siempre demostrar interés en lo que hago. Y por último gracias a todas **mis primas, Carol, Laura, Nohemí y Raquel.** Os deseo lo mejor en vuestras vida y a vuestras familias!

From the bottom of my heart, thanks to all my "**mousies**" that have made possible all the work in this thesis, they made this work so valuable and fruitful. Thank you.

6 REFERENCES

1. McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 2016, 7(2): 418-419.

2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011, 144(5): 646-674.

3. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F, et al. International incidence of childhood cancer, 2001-10: a population-based registry study. Lancet Oncol 2017, 18(6): 719-731.

4. Scotting PJ, Walker DA, Perilongo G. Childhood solid tumours: a developmental disorder. Nat Rev Cancer 2005, 5(6): 481-488.

5. Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. N Engl J Med 2015, 373(24): 2336-2346.

6. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013, 500(7463): 415-421.

7. Mulhern RK, Merchant TE, Gajjar A, Reddick WE, Kun LE. Late neurocognitive sequelae in survivors of brain tumours in childhood. Lancet Oncol 2004, 5(7): 399-408.

8. Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. Nat Rev Cancer 2006, 6(9): 702-713.

9. Maris JM. Recent advances in neuroblastoma. N Engl J Med 2010, 362(23): 2202-2211.

10. Brodeur GM, Seeger RC, Barrett A, Castleberry RP, D'Angio G, De Bernardi B, et al. International criteria for diagnosis, staging and response to treatment in patients with neuroblastoma. Prog Clin Biol Res 1988, 271: 509-524.

11. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993, 11(8): 1466-1477.

12. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol 2009, 27(2): 289-297.

13. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer 1999, 86(2): 364-372.

14. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 2003, 3(3): 203-216.

15. Johnsen JI, Kogner P, Albihn A, Henriksson MA. Embryonal neural tumours and cell death. Apoptosis 2009, 14(4): 424-438.

16. Jiang M, Stanke J, Lahti JM. The connections between neural crest development and neuroblastoma. Curr Top Dev Biol 2011, 94: 77-127.

17. General Introduction. In: Kalcheim C, Le Douarin N (eds). The Neural Crest, 2 edn. Cambridge University Press: Cambridge, 1999, pp xxi-xxiv.

18. The Migration of Neural Crest Cells. In: Kalcheim C, Le Douarin N (eds). The Neural Crest, 2 edn. Cambridge University Press: Cambridge, 1999, pp 23-59.

19. Meulemans D, Bronner-Fraser M. Gene-regulatory interactions in neural crest evolution and development. Dev Cell 2004, 7(3): 291-299.

20. Ernsberger U, Esposito L, Partimo S, Huber K, Franke A, Bixby JL, et al. Expression of neuronal markers suggests heterogeneity of chick sympathoadrenal cells prior to invasion of the adrenal anlagen. Cell Tissue Res 2005, 319(1): 1-13.

21. Howard MJ, Stanke M, Schneider C, Wu X, Rohrer H. The transcription factor dHAND is a downstream effector of BMPs in sympathetic neuron specification. Development 2000, 127(18): 4073-4081.

22. Reissmann E, Ernsberger U, Francis-West PH, Rueger D, Brickell PM, Rohrer H. Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. Development 1996, 122(7): 2079-2088.

23. Schneider C, Wicht H, Enderich J, Wegner M, Rohrer H. Bone morphogenetic proteins are required in vivo for the generation of sympathetic neurons. Neuron 1999, 24(4): 861-870.

24. Maris JM, Kyemba SM, Rebbeck TR, White PS, Sulman EP, Jensen SJ, et al. Molecular genetic analysis of familial neuroblastoma. Eur J Cancer 1997, 33(12): 1923-1928.

25. Janoueix-Lerosey I, Lopez-Delisle L, Delattre O, Rohrer H. The ALK receptor in sympathetic neuron development and neuroblastoma. Cell Tissue Res 2018, 372(2): 325-337.

26. Pattyn A, Morin X, Cremer H, Goridis C, Brunet J-F. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 1999, 399: 366.

27. Tiveron MC, Hirsch MR, Brunet JF. The expression pattern of the transcription factor Phox2 delineates synaptic pathways of the autonomic nervous system. J Neurosci 1996, 16(23): 7649-7660.

28. Bourdeaut F, Trochet D, Janoueix-Lerosey I, Ribeiro A, Deville A, Coz C, et al. Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. Cancer Lett 2005, 228(1-2): 51-58.

29. Mosse YP, Laudenslager M, Khazi D, Carlisle AJ, Winter CL, Rappaport E, et al. Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet 2004, 75(4): 727-730.

30. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008, 455(7215): 930-935.

31. Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev 2000, 14(23): 2919-2937.

32. Hubbard SR, Miller WT. Receptor tyrosine kinases: mechanisms of activation and signaling. Current opinion in cell biology 2007, 19(2): 117-123.

33. Stoica GE, Kuo A, Aigner A, Sunitha I, Souttou B, Malerczyk C, et al. Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. J Biol Chem 2001, 276(20): 16772-16779.

34. Iwahara T, Fujimoto J, Wen D, Cupples R, Bucay N, Arakawa T, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene 1997, 14(4): 439-449.

35. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. Nature Reviews Cancer 2013, 13: 685.

36. Weiss JB, Xue C, Benice T, Xue L, Morris SW, Raber J. Anaplastic lymphoma kinase and leukocyte tyrosine kinase: functions and genetic interactions in learning, memory and adult neurogenesis. Pharmacol Biochem Behav 2012, 100(3): 566-574.

37. Witek B, El Wakil A, Nord C, Ahlgren U, Eriksson M, Vernersson-Lindahl E, et al. Targeted Disruption of ALK Reveals a Potential Role in Hypogonadotropic Hypogonadism. PLoS One 2015, 10(5): e0123542.

38. Morris SW, Kirstein MN, Valentine MB, Dittmer K, Shapiro DN, Look AT, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1995, 267(5196): 316-317.

39. Hallberg B, Palmer RH. The role of the ALK receptor in cancer biology. Ann Oncol 2016, 27 Suppl 3: iii4-iii15.

40. Caren H, Abel F, Kogner P, Martinsson T. High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. Biochem J 2008, 416(2): 153-159.

41. Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature 2008, 455(7215): 971-974.

42. Mosse YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008, 455(7215): 930-935.

43. Janoueix-Lerosey I, Lequin D, Brugieres L, Ribeiro A, de Pontual L, Combaret V, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 2008, 455(7215): 967-970.

44. Bresler SC, Weiser DA, Huwe PJ, Park JH, Krytska K, Ryles H, et al. ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. Cancer Cell 2014, 26(5): 682-694.

45. Schleiermacher G, Javanmardi N, Bernard V, Leroy Q, Cappo J, Rio Frio T, et al. Emergence of new ALK mutations at relapse of neuroblastoma. J Clin Oncol 2014, 32(25): 2727-2734.

46. Zhu S, Lee J-S, Guo F, Shin J, Perez-Atayde AR, Kutok JL, et al. Activated ALK collaborates with MYCN in neuroblastoma pathogenesis. Cancer cell 2012, 21(3): 362-373.

47. De Brouwer S, De Preter K, Kumps C, Zabrocki P, Porcu M, Westerhout EM, et al. Meta-analysis of neuroblastomas reveals a skewed ALK mutation spectrum in tumors with MYCN amplification. Clin Cancer Res 2010, 16(17): 4353-4362.

48. Berry T, Luther W, Bhatnagar N, Jamin Y, Poon E, Sanda T, et al. The ALK(F1174L) mutation potentiates the oncogenic activity of MYCN in neuroblastoma. Cancer Cell 2012, 22(1): 117-130.

49. Seitz G, Armeanu-Ebinger S, Warmann S, Fuchs J. Animal models of extracranial pediatric solid tumors. Oncol Lett 2012, 4(5): 859-864.

50. Huang M, Weiss WA. Neuroblastoma and MYCN. Cold Spring Harb Perspect Med 2013, 3(10): a014415.

51. Weiss WA, Aldape K, Mohapatra G, Feuerstein BG, Bishop JM. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J 1997, 16(11): 2985-2995.

52. Althoff K, Beckers A, Bell E, Nortmeyer M, Thor T, Sprussel A, et al. A Cre-conditional MYCN-driven neuroblastoma mouse model as an improved tool for preclinical studies. Oncogene 2015, 34(26): 3357-3368.

53. Moore HC, Wood KM, Jackson MS, Lastowska MA, Hall D, Imrie H, et al. Histological profile of tumours from MYCN transgenic mice. J Clin Pathol 2008, 61(10): 1098-1103.

54. Braekeveldt N, Wigerup C, Gisselsson D, Mohlin S, Merselius M, Beckman S, et al. Neuroblastoma patient-derived orthotopic xenografts retain metastatic patterns and geno- and phenotypes of patient tumours. Int J Cancer 2015, 136(5): E252-261.

55. Johnson KJ, Cullen J, Barnholtz-Sloan JS, Ostrom QT, Langer CE, Turner MC, et al. Childhood brain tumor epidemiology: a brain tumor epidemiology consortium review. Cancer Epidemiol Biomarkers Prev 2014, 23(12): 2716-2736.

56. Palmer SL. Neurodevelopmental impact on children treated for medulloblastoma: a review and proposed conceptual model. Developmental disabilities research reviews 2008, 14(3): 203-210.

57. DeSouza RM, Jones BR, Lowis SP, Kurian KM. Pediatric medulloblastoma - update on molecular classification driving targeted therapies. Front Oncol 2014, 4: 176.

58. Palmer SL. Neurodevelopmental impact on children treated for medulloblastoma: a review and proposed conceptual model. Dev Disabil Res Rev 2008, 14(3): 203-210.

59. Rousseau A, Mokhtari K, Duyckaerts C. The 2007 WHO classification of tumors of the central nervous system - what has changed? Curr Opin Neurol 2008, 21(6): 720-727.

60. Eberhart CG, Burger PC. Anaplasia and grading in medulloblastomas. Brain Pathol 2003, 13(3): 376-385.

61. Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. Nat Rev Neurol 2012, 8(6): 340-351.

62. Eberhart CG, Kepner JL, Goldthwaite PT, Kun LE, Duffner PK, Friedman HS, et al. Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study. Cancer 2002, 94(2): 552-560.

63. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol 2011, 29(11): 1408-1414.

64. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol 2012, 123(4): 465-472.

65. Tailor J, Kittappa R, Leto K, Gates M, Borel M, Paulsen O, et al. Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency. J Neurosci 2013, 33(30): 12407-12422.

66. Cavalli FMG, Remke M, Rampasek L, Peacock J, Shih DJH, Luu B, et al. Intertumoral Heterogeneity within Medulloblastoma Subgroups. Cancer Cell 2017, 31(6): 737-754.e736.

67. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, et al. Subtypes of medulloblastoma have distinct developmental origins. Nature 2010, 468: 1095.

68. Ellison DW, Onilude OE, Lindsey JC, Lusher ME, Weston CL, Taylor RE, et al. beta-catenin status predicts a favorable outcome in childhood medulloblastoma: The United Kingdom Children's Cancer Study Group Brain Tumour Committee. Journal of Clinical Oncology 2005, 23(31): 7951-7957.

69. Villani A, Malkin D, Tabori U. Syndromes predisposing to pediatric central nervous system tumors: lessons learned and new promises. Curr Neurol Neurosci Rep 2012, 12(2): 153-164.

70. Clifford SC, Lusher ME, Lindsey JC, Langdon JA, Gilbertson RJ, Straughton D, et al. Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct

molecular sub-group of medulloblastomas associated with a favorable prognosis. Cell Cycle 2006, 5(22): 2666-2670.

71. Zurawel RH, Allen C, Chiappa S, Cato W, Biegel J, Cogen P, et al. Analysis of PTCH/SMO/SHH pathway genes in medulloblastoma. Genes Chromosomes Cancer 2000, 27(1): 44-51.

72. Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, et al. Mutations in SUFU predispose to medulloblastoma. Nat Genet 2002, 31(3): 306-310.

73. Slade I, Murray A, Hanks S, Kumar A, Walker L, Hargrave D, et al. Heterogeneity of familial medulloblastoma and contribution of germline PTCH1 and SUFU mutations to sporadic medulloblastoma. Fam Cancer 2011, 10(2): 337-342.

74. Northcott PA, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, et al. Medulloblastomics: the end of the beginning. Nat Rev Cancer 2012, 12(12): 818-834.

75. Martinez S, Andreu A, Mecklenburg N, Echevarria D. Cellular and molecular basis of cerebellar development. Front Neuroanat 2013, 7: 18.

76. Wingate RJ. The rhombic lip and early cerebellar development. Curr Opin Neurobiol 2001, 11(1): 82-88.

77. Hatten ME, Heintz N. Mechanisms of neural patterning and specification in the developing cerebellum. Annu Rev Neurosci 1995, 18: 385-408.

78. Marino S. Medulloblastoma: developmental mechanisms out of control. Trends Mol Med 2005, 11(1): 17-22.

79. Rimkus TK, Carpenter RL, Qasem S, Chan M, Lo HW. Targeting the Sonic Hedgehog Signaling Pathway: Review of Smoothened and GLI Inhibitors. Cancers (Basel) 2016, 8(2).

80. Wenger SL, Senft JR, Sargent LM, Bamezai R, Bairwa N, Grant SG. Comparison of Established Cell Lines at Different Passages by Karyotype and Comparative Genomic Hybridization. Bioscience Reports 2004, 24(6): 631-639.

81. Jacobsen PF, Jenkyn DJ, Papadimitriou JM. Establishment of a human medulloblastoma cell line and its heterotransplantation into nude mice. J Neuropathol Exp Neurol 1985, 44(5): 472-485.

82. Keles GE, Berger MS, Srinivasan J, Kolstoe DD, Bobola MS, Silber JR. Establishment and characterization of four human medulloblastoma-derived cell lines. Oncol Res 1995, 7(10-11): 493-503.

83. Ivanov DP, Coyle B, Walker DA, Grabowska AM. In vitro models of medulloblastoma: Choosing the right tool for the job. J Biotechnol 2016, 236: 10-25.

84. Wu X, Northcott PA, Croul S, Taylor MD. Mouse models of medulloblastoma. Chin J Cancer 2011, 30(7): 442-449.

85. Pöschl J, Stark S, Neumann P, Gröbner S, Kawauchi D, Jones DTW, et al. Genomic and transcriptomic analyses match medulloblastoma mouse models to their human counterparts. Acta Neuropathologica 2014, 128(1): 123-136.

86. Park HL, Bai C, Platt KA, Matise MP, Beeghly A, Hui CC, et al. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. Development 2000, 127(8): 1593.

87. Li L, Connelly MC, Wetmore C, Curran T, Morgan JI. Mouse embryos cloned from brain tumors. Cancer Res 2003, 63(11): 2733-2736.

88. Kawauchi D, Robinson G, Uziel T, Gibson P, Rehg J, Gao C, et al. A mouse model of the most aggressive subgroup of human medulloblastoma. Cancer Cell 2012, 21(2): 168-180.

89. Wamaitha SE, Niakan KK. Chapter Twelve - Human Pre-gastrulation Development. In: Plusa B, Hadjantonakis A-K (eds). Current Topics in Developmental Biology, vol. 128. Academic Press, 2018, pp 295-338.

90. Shyamala K, Yanduri S, Girish HC, Murgod S. Neural crest: The fourth germ layer. J Oral Maxillofac Pathol 2015, 19(2): 221-229.

91. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981, 292: 154.

92. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 1981, 78(12): 7634-7638.

93. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998, 282(5391): 1145-1147.

94. Bongso A, Fong CY, Ng SC, Ratnam S. Isolation and culture of inner cell mass cells from human blastocysts. Hum Reprod 1994, 9(11): 2110-2117.

95. Hovatta O, Mikkola M, Gertow K, Stromberg AM, Inzunza J, Hreinsson J, et al. A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. Hum Reprod 2003, 18(7): 1404-1409.

96. Vazin T, Freed WJ. Human embryonic stem cells: derivation, culture, and differentiation: a review. Restor Neurol Neurosci 2010, 28(4): 589-603.

97. Cai J, Chen J, Liu Y, Miura T, Luo Y, Loring JF, et al. Assessing self-renewal and differentiation in human embryonic stem cell lines. Stem Cells 2006, 24(3): 516-530.

98. Andrews PW, Damjanov I, Simon D, Banting GS, Carlin C, Dracopoli NC, et al. Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. Lab Invest 1984, 50(2): 147-162.

99. Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirie F, Rigby PWJ, et al. A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 1990, 345: 686.

100. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 2000, 24(4): 372-376.

101. Zhou HY, Katsman Y, Dhaliwal NK, Davidson S, Macpherson NN, Sakthidevi M, et al. A Sox2 distal enhancer cluster regulates embryonic stem cell differentiation potential. Genes Dev 2014, 28(24): 2699-2711.

102. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 2003, 113(5): 631-642.

103. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morphol 1962, 10: 622-640.

104. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006, 126(4): 663-676.

105. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007, 131(5): 861-872.

106. Hackett CH, Fortier LA. Embryonic stem cells and iPS cells: sources and characteristics. Vet Clin North Am Equine Pract 2011, 27(2): 233-242.

107. Hochedlinger K, Jaenisch R. Nuclear reprogramming and pluripotency. Nature 2006, 441(7097): 1061-1067.

108. Gonzalez F, Boue S, Izpisua Belmonte JC. Methods for making induced pluripotent stem cells: reprogramming a la carte. Nat Rev Genet 2011, 12(4): 231-242.

109. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008, 322(5903): 949-953.

110. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proceedings of the Japan Academy Series B, Physical and biological sciences 2009, 85(8): 348-362.

111. Rosa A, Brivanlou AH. Synthetic mRNAs: powerful tools for reprogramming and differentiation of human cells. Cell Stem Cell 2010, 7(5): 549-550.

112. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 1998, 95(3): 379-391.

113. Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, et al. Nanog safeguards pluripotency and mediates germline development. Nature 2007, 450(7173): 1230-1234.

114. Rowland BD, Bernards R, Peeper DS. The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. Nat Cell Biol 2005, 7(11): 1074-1082.

115. Zhou Q, Hong Y, Zhan Q, Shen Y, Liu Z. Role for Kruppel-like factor 4 in determining the outcome of p53 response to DNA damage. Cancer Res 2009, 69(21): 8284-8292.

116. Rasmussen MA, Holst B, Tümer Z, Johnsen MG, Zhou S, Stummann TC, et al. Transient p53 suppression increases reprogramming of human fibroblasts without affecting apoptosis and DNA damage. Stem cell reports 2014, 3(3): 404-413.

117. Omole AE, Fakoya AOJ. Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. PeerJ 2018, 6: e4370.

118. Buganim Y, Faddah DA, Jaenisch R. Mechanisms and models of somatic cell reprogramming. Nat Rev Genet 2013, 14(6): 427-439.

119. Liang G, Zhang Y. Embryonic stem cell and induced pluripotent stem cell: an epigenetic perspective. Cell Res 2013, 23(1): 49-69.

120. Chen KG, Mallon BS, McKay RD, Robey PG. Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics. Cell Stem Cell 2014, 14(1): 13-26.

121. Genbacev O, Krtolica A, Zdravkovic T, Brunette E, Powell S, Nath A, et al. Serum-free derivation of human embryonic stem cell lines on human placental fibroblast feeders. Fertil Steril 2005, 83(5): 1517-1529.

122. Li Y, Powell S, Brunette E, Lebkowski J, Mandalam R. Expansion of human embryonic stem cells in defined serum-free medium devoid of animal-derived products. Biotechnol Bioeng 2005, 91(6): 688-698.

123. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, et al. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol 2006, 24(2): 185-187.

124. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, et al. Feeder-free growth of undifferentiated human embryonic stem cells. Nat Biotechnol 2001, 19(10): 971-974.

125. Belkin AM, Stepp MA. Integrins as receptors for laminins. Microsc Res Techniq 2000, 51(3): 280-301.

126. Rodin S, Antonsson L, Niaudet C, Simonson OE, Salmela E, Hansson EM, et al. Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nature Communications 2014, 5: 3195.

127. Rodin S, Domogatskaya A, Strom S, Hansson EM, Chien KR, Inzunza J, et al. Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. Nat Biotechnol 2010, 28(6): 611-615.

128. Buzzard JJ, Gough NM, Crook JM, Colman A. Karyotype of human ES cells during extended culture. Nat Biotechnol 2004, 22(4): 381-382; author reply 382.

129. Merkle FT, Ghosh S, Kamitaki N, Mitchell J, Avior Y, Mello C, et al. Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. Nature 2017, 545(7653): 229-+.

130. Fong H, Hohenstein KA, Donovan PJ. Regulation of self-renewal and pluripotency by Sox2 in human embryonic stem cells. Stem Cells 2008, 26(8): 1931-1938.

131. Pan G, Thomson JA. Nanog and transcriptional networks in embryonic stem cell pluripotency. Cell Res 2007, 17(1): 42-49.

132. Müller F-J, Schuldt BM, Williams R, Mason D, Altun G, Papapetrou EP, et al. A bioinformatic assay for pluripotency in human cells. Nature Methods 2011, 8: 315.

133. Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 2014, 6(2): 114-118.

134. Sandberg R, Ernberg I. Assessment of tumor characteristic gene expression in cell lines using a tissue similarity index (TSI). Proc Natl Acad Sci U S A 2005, 102(6): 2052-2057.

135. Kumar S, Blangero J, Curran JE. Induced Pluripotent Stem Cells in Disease Modeling and Gene Identification. Methods Mol Biol 2018, 1706: 17-38.

136. Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, et al. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 2011, 8(3): 267-280.

137. Narsinh K, Narsinh KH, Wu JC. Derivation of human induced pluripotent stem cells for cardiovascular disease modeling. Circ Res 2011, 108(9): 1146-1156.

138. Ross PJ, Ellis J. Modeling complex neuropsychiatric disease with induced pluripotent stem cells. F1000 Biol Rep 2010, 2: 84.

139. Marin Navarro A, Susanto E, Falk A, Wilhelm M. Modeling cancer using patient-derived induced pluripotent stem cells to understand development of childhood malignancies. Cell death discovery 2018, 4(1): 7.

140. Carette JE, Pruszak J, Varadarajan M, Blomen VA, Gokhale S, Camargo FD, et al. Generation of iPSCs from cultured human malignant cells. Blood 2010, 115(20): 4039-4042.

141. Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, et al. Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. Blood 2012, 119(26): 6234-6242.

142. Miyoshi N, Ishii H, Nagai K, Hoshino H, Mimori K, Tanaka F, et al. Defined factors induce reprogramming of gastrointestinal cancer cells. Proc Natl Acad Sci U S A 2010, 107(1): 40-45.

143. Kim J, Hoffman JP, Alpaugh RK, Rhim AD, Reichert M, Stanger BZ, et al. An iPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. Cell reports 2013, 3(6): 2088-2099.

144. Kim J, Zaret KS. Reprogramming of human cancer cells to pluripotency for models of cancer progression. EMBO J 2015, 34(6): 739-747.

145. Ohnishi K, Semi K, Yamamoto T, Shimizu M, Tanaka A, Mitsunaga K, et al. Premature termination of reprogramming in vivo leads to cancer development through altered epigenetic regulation. Cell 2014, 156(4): 663-677.

146. Rahman N. Realizing the promise of cancer predisposition genes. Nature 2014, 505(7483): 302-308.

147. Lee DF, Su J, Kim HS, Chang B, Papatsenko D, Zhao R, et al. Modeling familial cancer with induced pluripotent stem cells. Cell 2015, 161(2): 240-254.

148. Trochet D, Bourdeaut F, Janoueix-Lerosey I, Deville A, de Pontual L, Schleiermacher G, et al. Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. Am J Hum Genet 2004, 74(4): 761-764.

149. Gotz M, Huttner WB. The cell biology of neurogenesis. Nat Rev Mol Cell Biol 2005, 6(10): 777-788.

150. Mora-Bermúdez F, García MT, Huttner WB. Stem Cells: Neural Stem Cells in Cerebral Cortex Development. In: Pfaff DW (ed). Neuroscience in the 21st Century: From Basic to Clinical. Springer New York: New York, NY, 2013, pp 137-159.

151. Wilson SI, Edlund T. Neural induction: toward a unifying mechanism. Nat Neurosci 2001, 4 Suppl: 1161-1168.

152. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 2001, 19(12): 1129-1133.

153. Elkabetz Y, Panagiotakos G, Al Shamy G, Socci ND, Tabar V, Studer L. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. Genes Dev 2008, 22(2): 152-165.

154. Koch P, Opitz T, Steinbeck JA, Ladewig J, Brustle O. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc Natl Acad Sci U S A 2009, 106(9): 3225-3230.

155. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol 2009, 27(3): 275-280.

156. Ciccolini F, Svendsen CN. Fibroblast Growth Factor 2 (FGF-2) Promotes Acquisition of Epidermal Growth Factor (EGF) Responsiveness in Mouse Striatal Precursor Cells: Identification of Neural Precursors Responding to both EGF and FGF-2. The Journal of Neuroscience 1998, 18(19): 7869.

157. Simões-Costa M, Bronner ME. Insights into neural crest development and evolution from genomic analysis. Genome research 2013, 23(7): 1069-1080.

158. Sauka-Spengler T, Bronner-Fraser M. A gene regulatory network orchestrates neural crest formation. Nat Rev Mol Cell Biol 2008, 9(7): 557-568.

159. Kleber M, Lee HY, Wurdak H, Buchstaller J, Riccomagno MM, Ittner LM, et al. Neural crest stem cell maintenance by combinatorial Wnt and BMP signaling. J Cell Biol 2005, 169(2): 309-320.

160. Wilson PA, Hemmati-Brivanlou A. Vertebrate neural induction: inducers, inhibitors, and a new synthesis. Neuron 1997, 18(5): 699-710.

161. Mayor R, Aybar MJ. Induction and development of neural crest in Xenopus laevis. Cell Tissue Res 2001, 305(2): 203-209.

162. LaBonne C, Bronner-Fraser M. Neural crest induction in Xenopus: evidence for a two-signal model. Development 1998, 125(13): 2403-2414.

163. Menendez L, Kulik MJ, Page AT, Park SS, Lauderdale JD, Cunningham ML, et al. Directed differentiation of human pluripotent cells to neural crest stem cells. Nat Protoc 2013, 8(1): 203-212.

164. García-Castro MnI, Marcelle C, Bronner-Fraser M. Ectodermal Wnt Function as a Neural Crest Inducer. Science 2002, 297(5582): 848.

165. Lee G, Chambers SM, Tomishima MJ, Studer L. Derivation of neural crest cells from human pluripotent stem cells. Nat Protoc 2010, 5(4): 688-701.

166. Menendez L, Yatskievych TA, Antin PB, Dalton S. Wnt signaling and a Smad pathway blockade direct the differentiation of human pluripotent stem cells to multipotent neural crest cells. Proc Natl Acad Sci U S A 2011, 108(48): 19240-19245.

167. Mica Y, Lee G, Chambers SM, Tomishima MJ, Studer L. Modeling neural crest induction, melanocyte specification, and disease-related pigmentation defects in hESCs and patient-specific iPSCs. Cell Rep 2013, 3(4): 1140-1152.

168. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. Nature 2013, 501(7467): 373-379.

169. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt–villus structures in vitro without a mesenchymal niche. Nature 2009, 459: 262.

170. Clevers H. Modeling Development and Disease with Organoids. Cell 2016, 165(7): 1586-1597.

171. Taverna E, Huttner WB. Neural progenitor nuclei IN motion. Neuron 2010, 67(6): 906-914.

172. Götz M, Huttner WB. The cell biology of neurogenesis. Nature Reviews Molecular Cell Biology 2005, 6: 777.

173. Pollen Alex A, Nowakowski Tomasz J, Chen J, Retallack H, Sandoval-Espinosa C, Nicholas Cory R, et al. Molecular Identity of Human Outer Radial Glia during Cortical Development. Cell 2015, 163(1): 55-67.

174. Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 2004, 7(2): 136-144.

175. Lui JH, Hansen DV, Kriegstein AR. Development and evolution of the human neocortex. Cell 2011, 146(1): 18-36.

176. Florio M, Huttner WB. Neural progenitors, neurogenesis and the evolution of the neocortex. Development 2014, 141(11): 2182-2194.

177. Espuny-Camacho I, Michelsen KA, Gall D, Linaro D, Hasche A, Bonnefont J, et al. Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron 2013, 77(3): 440-456.

178. Ying Q-L, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nature Biotechnology 2003, 21: 183.

179. Watanabe K, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, et al. Directed differentiation of telencephalic precursors from embryonic stem cells. Nat Neurosci 2005, 8(3): 288-296.

180. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 2008, 3(5): 519-532.

181. Mariani J, Simonini MV, Palejev D, Tomasini L, Coppola G, Szekely AM, et al. Modeling human cortical development in vitro using induced pluripotent stem cells. Proceedings of the National Academy of Sciences 2012, 109(31): 12770.

182. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009, 459(7244): 262-265.

183. Stange DE, Koo BK, Huch M, Sibbel G, Basak O, Lyubimova A, et al. Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. Cell 2013, 155(2): 357-368.

184. Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, et al. In vitro generation of human pluripotent stem cell derived lung organoids. Elife 2015, 4.

185. DeWard AD, Cramer J, Lagasse E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. Cell Rep 2014, 9(2): 701-711.

186. Kelava I, Lancaster MA. Dishing out mini-brains: Current progress and future prospects in brain organoid research. Dev Biol 2016, 420(2): 199-209.

187. Bourdon JC. p53 Family isoforms. Curr Pharm Biotechnol 2007, 8(6): 332-336.

188. Belyi VA, Ak P, Markert E, Wang H, Hu W, Puzio-Kuter A, et al. The origins and evolution of the p53 family of genes. Cold Spring Harb Perspect Biol 2010, 2(6): a001198.

189. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. Annu Rev Biochem 2008, 77: 557-582.

190. Khoury MP, Bourdon JC. The isoforms of the p53 protein. Cold Spring Harb Perspect Biol 2010, 2(3): a000927.

191. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000, 408(6810): 307-310.

192. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 1993, 75(4): 805-816.

193. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995, 80(2): 293-299.

194. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001, 7(3): 683-694.

195. Shinmura K, Bennett RA, Tarapore P, Fukasawa K. Direct evidence for the role of centrosomally localized p53 in the regulation of centrosome duplication. Oncogene 2007, 26(20): 2939-2944.

196. Puzio-Kuter AM. The Role of p53 in Metabolic Regulation. Genes Cancer 2011, 2(4): 385-391.

197. Menendez S, Camus S, Izpisua Belmonte JC. p53: guardian of reprogramming. Cell Cycle 2010, 9(19): 3887-3891.

198. Lane DP. Cancer. p53, guardian of the genome. Nature 1992, 358(6381): 15-16.

199. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedback loop. Genes Dev 1993, 7(7A): 1126-1132.

200. Van Nostrand JL, Brady CA, Jung H, Fuentes DR, Kozak MM, Johnson TM, et al. Inappropriate p53 activation during development induces features of CHARGE syndrome. Nature 2014, 514(7521): 228-232.

201. Shin MH, He Y, Huang J. Embryonic stem cells shed new light on the developmental roles of p53. Cell Biosci 2013, 3(1): 42.

202. Sah VP, Attardi LD, Mulligan GJ, Williams BO, Bronson RT, Jacks T. A subset of p53-deficient embryos exhibit exencephaly. Nat Genet 1995, 10(2): 175-180.

203. Armstrong JF, Kaufman MH, Harrison DJ, Clarke AR. High-frequency developmental abnormalities in p53-deficient mice. Curr Biol 1995, 5(8): 931-936.

204. Schmid P, Lorenz A, Hameister H, Montenarh M. Expression of p53 during mouse embryogenesis. Development 1991, 113(3): 857-865.

205. Fulci G, Van Meir EG. p53 and the CNS. Molecular Neurobiology 1999, 19(1): 61-77.

206. Komarova EA, Chernov MV, Franks R, Wang K, Armin G, Zelnick CR, et al. Transgenic mice with p53-responsive lacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity in vivo. EMBO J 1997, 16(6): 1391-1400.

207. Gottlieb E, Haffner R, King A, Asher G, Gruss P, Lonai P, et al. Transgenic mouse model for studying the transcriptional activity of the p53 protein: age- and tissue-dependent changes in radiation-induced activation during embryogenesis. EMBO J 1997, 16(6): 1381-1390.

208. Xiong S, Van Pelt CS, Elizondo-Fraire AC, Liu G, Lozano G. Synergistic roles of Mdm2 and Mdm4 for p53 inhibition in central nervous system development. Proc Natl Acad Sci U S A 2006, 103(9): 3226-3231.

209. Meletis K, Wirta V, Hede SM, Nister M, Lundeberg J, Frisen J. p53 suppresses the self-renewal of adult neural stem cells. Development 2006, 133(2): 363-369.

210. Armesilla-Diaz A, Bragado P, Del Valle I, Cuevas E, Lazaro I, Martin C, et al. p53 regulates the self-renewal and differentiation of neural precursors. Neuroscience 2009, 158(4): 1378-1389.

211. Liu H, Jia D, Li A, Chau J, He D, Ruan X, et al. p53 regulates neural stem cell proliferation and differentiation via BMP-Smad1 signaling and Id1. Stem Cells Dev 2013, 22(6): 913-927.

212. Marin Navarro A, Day K, Kogner P, Wilhelm M, Falk A. Generation of induced pluripotent stem cell lines from two Neuroblastoma patients carrying a germline ALK R1275Q mutation. Stem Cell Research 2019, 34: 101356.

213. Hackland JOS, Frith TJR, Thompson O, Marin Navarro A, Garcia-Castro MI, Unger C, et al. Top-Down Inhibition of BMP Signaling Enables Robust Induction of hPSCs Into Neural Crest in Fully Defined, Xeno-free Conditions. Stem Cell Reports 2017, 9(4): 1043-1052.

214. Uhlin E, Marin Navarro A, Ronnholm H, Day K, Kele M, Falk A. Integration Free Derivation of Human Induced Pluripotent Stem Cells Using Laminin 521 Matrix. J Vis Exp 2017(125).

215. Banito A, Rashid ST, Acosta JC, Li S, Pereira CF, Geti I, et al. Senescence impairs successful reprogramming to pluripotent stem cells. Genes Dev 2009, 23(18): 2134-2139.

216. Rodin S, Antonsson L, Hovatta O, Tryggvason K. Monolayer culturing and cloning of human pluripotent stem cells on laminin-521-based matrices under xeno-free and chemically defined conditions. Nat Protoc 2014, 9(10): 2354-2368.

217. Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev Biol 2000, 227(2): 271-278.

218. Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, et al. Chemically defined conditions for human iPSC derivation and culture. Nat Methods 2011, 8(5): 424-429.

219. Rodin S, Antonsson L, Niaudet C, Simonson OE, Salmela E, Hansson EM, et al. Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nat Commun 2014, 5: 3195.

220. Basch ML, Bronner-Fraser M, Garcia-Castro MI. Specification of the neural crest occurs during gastrulation and requires Pax7. Nature 2006, 441(7090): 218-222.

221. Betters E, Liu Y, Kjaeldgaard A, Sundstrom E, Garcia-Castro MI. Analysis of early human neural crest development. Dev Biol 2010, 344(2): 578-592.

222. Lamb TM, Knecht AK, Smith WC, Stachel SE, Economides AN, Stahl N, et al. Neural induction by the secreted polypeptide noggin. Science 1993, 262(5134): 713-718.

223. Pera MF, Andrade J, Houssami S, Reubinoff B, Trounson A, Stanley EG, et al. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J Cell Sci 2004, 117(Pt 7): 1269-1280.

224. Tremblay P, Kessel M, Gruss P. A transgenic neuroanatomical marker identifies cranial neural crest deficiencies associated with the Pax3 mutant Splotch. Dev Biol 1995, 171(2): 317-329.

225. Southard-Smith EM, Kos L, Pavan WJ. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nat Genet 1998, 18(1): 60-64.

226. Zhang J, Hagopian-Donaldson S, Serbedzija G, Elsemore J, Plehn-Dujowich D, McMahon AP, et al. Neural tube, skeletal and body wall defects in mice lacking transcription factor AP-2. Nature 1996, 381(6579): 238-241.

227. Kele M, Day K, Ronnholm H, Schuster J, Dahl N, Falk A. Generation of human iPS cell line CTL07-II from human fibroblasts, under defined and xeno-free conditions. Stem Cell Res 2016, 17(3): 474-478.

228. Lonergan GJ, Schwab CM, Suarez ES, Carlson CL. Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. Radiographics 2002, 22(4): 911-934.

229. Falk A, Koch P, Kesavan J, Takashima Y, Ladewig J, Alexander M, et al. Capture of neuroepithelial-like stem cells from pluripotent stem cells provides a versatile system for in vitro production of human neurons. PLoS One 2012, 7(1): e29597.

230. Sasai K, Romer JT, Lee Y, Finkelstein D, Fuller C, McKinnon PJ, et al. Shh pathway activity is down-regulated in cultured medulloblastoma cells: implications for preclinical studies. Cancer Res 2006, 66(8): 4215-4222.

231. Luo C, Lancaster MA, Castanon R, Nery JR, Knoblich JA, Ecker JR. Cerebral Organoids Recapitulate Epigenomic Signatures of the Human Fetal Brain. Cell Reports 2016, 17(12): 3369-3384.

232. Uhlin E, Ronnholm H, Day K, Kele M, Tammimies K, Bolte S, et al. Derivation of human iPS cell lines from monozygotic twins in defined and xeno free conditions. Stem Cell Res 2017, 18: 22-25.

233. Ellis P, Fagan BM, Magness ST, Hutton S, Taranova O, Hayashi S, et al. SOX2, a Persistent Marker for Multipotential Neural Stem Cells Derived from Embryonic Stem

Cells, the Embryo or the Adult. Developmental Neuroscience 2004, 26(2-4): 148-165.

234. Hutton SR, Pevny LH. SOX2 expression levels distinguish between neural progenitor populations of the developing dorsal telencephalon. Developmental Biology 2011, 352(1): 40-47.

235. Englund C, Fink A, Lau C, Pham D, Daza RAM, Bulfone A, et al. Pax6, Tbr2, and Tbr1 Are Expressed Sequentially by Radial Glia, Intermediate Progenitor Cells, and Postmitotic Neurons in Developing Neocortex. The Journal of Neuroscience 2005, 25(1): 247.

236. Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarro LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med 2017, 23(12): 1424-1435.

237. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015, 161(4): 933-945.