Pediatric Oncology: Looking for Similarities Between the Mechanisms Leading to Acute Lymphoblastic Leukemia and Neuroblastoma

Daniël M. van Herwijnen[1], Ianthe A.E.M. van Belzen[2], and Jayne Y. Hehir-Kwa[3]

[1]*Author*
[2]*Supervisor, Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands*
[3]*Supervisor, Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands,*
J.Y.HehirKwa@prinsesmaximacentrum.nl

Abstract

Adult cancers typically have a high mutational burden that has accumulated over time. In contrast, pediatric cancer has low mutational burden and the mechanisms driving pediatric cancer are still under-explored. acute lymphoblastic leukemia (ALL) and neuroblastoma (NB) are two of the most prevalent pediatric tumors, both having subgroups presenting with a high number of whole chromosome gains. This strong karyotypical resemblance could indicate that the tumors might share mechanisms. High Risk NB, however, is a very different disease and appears to be driven by *MYCN* amplification. Telomere shortening might be involved in the development of High Risk NB. In this review, mechanisms underlying ALL and NB have been compared to find similarities and differences that can potentially help to elucidate the underlying disease mechanisms. Both Low Risk NB and Hyperdiploid ALL could potentially be caused by a single erratic mitotic event, possibly multipolar division, although whole genome duplication followed by chromosome loss is more likely for NB. High Risk NB is characterized by *MYCN* amplification and it has been proposed that this might be caused by seismic amplification as a consequence of telomere shortening. In contrast, fusion-gene driven ALL are mostly caused by translocations and these tumors usually do not carry complex structural variations. This indicates that telomere shortening does not play a marked role in these tumors.

Keywords: acute lymphoblastic leukemia (ALL), neuroblastoma (NB), multipolar division, telomere shortening, structural variation, polyploidy, aneuploidy

1 Layman's summary

Most adult cancer is caused by acquiring mutations over time, but much less is known about the causes of childhood cancers. neuroblastoma and acute lymphoblastic leukemia are amongst the most common pediatric cancers. Both have a subgroup that present with very similar characteristics, which could indicate that they might share a mechanistic origin. In contrast, the High Risk subgroup of neuroblastoma has a poor outcome and is much different from Low Risk neuroblastoma. In this review, what is known about the mechanisms of these two types of pediatric cancer have been compared to find similarities and differences. These can help researchers to formulate new hypotheses to better study pediatric cancer. A mechanism these tumors might share is an error in cell division, causing the chromosomes to be incorrectly segregated. This results in number of chromosomes per cell deviating from the normal 46. However, it also seems likely that neuroblastoma (NB) is caused by whole genome duplication and subsequently loses chromosomes. The High Risk subgroup of neuroblastoma is driven by amplification of the oncogene *MYCN*. *MYCN* causes activation of telomerase, which causes telomere maintenance. Telomere maintenance is required to prevent the loss of DNA at the chromosome ends. It is unclear what causes *MYCN* amplification and it has been proposed that this is due to the shortening of telomeres, which causes chromosome fusion resulting in complex DNA damage.

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Corresponding author
Jayne Y. Hehir-Kwa

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Daniël M. van Herwijnen

2 Introduction

Pediatric and adult cancer are the leading cause of death in their respective demographic groups (Kattner *et al.* 2019). However, both diseases are quite different from each other. Typically, adult cancer is characterized by a high mutational burden due to accumulation of mutations over an extended period of time. In contrast, pediatric cancer generally lacks such a high mutational burden and instead has more gene-fusions, copy number alterations (CNAs) and structural variations (SVs) (Casey and Stewart 2020). CNAs have been found in acute lymphoblastic leukemia (ALL) and neuroblastoma (NB), two of the most prevalent types of pediatric cancer. The exact mechanisms that cause pediatric cancer are still poorly understood. Here, we compare similarities and differences between ALL and NB. This can lead to new insights into the underlying mechanisms of pediatric cancer development and ultimately to better treatment.

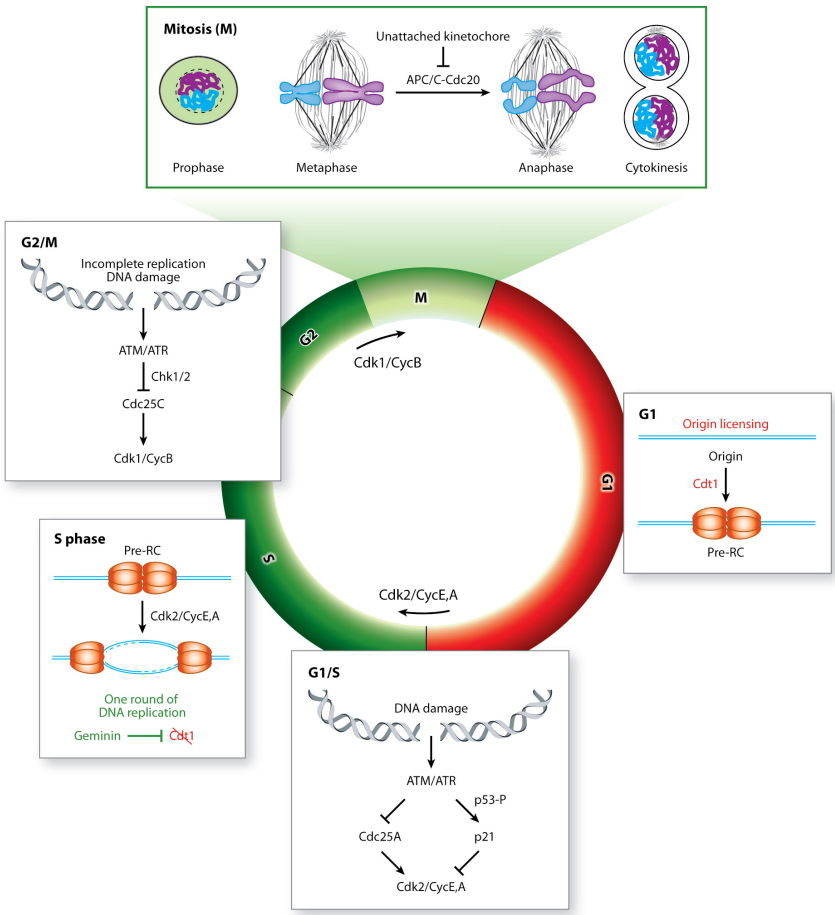
ALL is the most prevalent pediatric cancer with 26% of all cancer in children (Ward *et al.* 2014). The tumors arise from T- and B-cell precursors, in some cases already *in utero* (Panzer-Grümayer *et al.* 2002). ALL is amongst the top 3 of pediatric cancer survival (Ward *et al.* 2014) and has a high 5-year event-free and overall survival rate (Jeha *et al.* 2019). Subgroups of ALL are formed by the presence of specific gene-fusions, such as *BCR-ABL1*, MLL-rearrangements and *ETV6-RUNX1* (Arber *et al.* 2016), but the most prevalent form of ALL is Hyperdiploid ALL. An ALL is considered hyperdiploid when the tumor genome contains >50 chromosomes and is characterized by trisomy of chromosomes X, 4, 6, 10, 14, 17, 18 and tetrasomy 21 (Moura-Castro *et al.* 2021). Importantly, Hyperdiploid ALL appears to be chromosomally stable given the lack of subclones (Paulsson and Johansson 2009). Finally, ALL is further divided by chromosome count into hypodiploid, hyperdiploid, hypotriploid and hypertriploid (or near-triploid), and near-tetraploid (Haas and Borkhardt 2022). ALL characterized by gene-fusions is associated with poor outcome in contrast to Hyperdiploid ALL, which is considered a low risk group (Haas and Borkhardt 2022) and thereby displays similarities with Low Risk NB.

Neuroblastoma (NB) is the most frequent extracranial solid tumor in children and is are very heterogeneous disease in both characteristics and outcome (K. Campbell *et al.* 2023). Like Hyperdiploid ALL, Low Risk NB is characterized by near-triploidy and has a good prognosis (Brodeur and Bagatell 2014). Low Risk NB even has cases of spontaneous regression (Ackermann *et al.* 2018), although this is unlike ALL. In contrast, High Risk NB has a much worse outcome and is characterized by tetraploidy and segmental loss of chromosomes (Ackermann *et al.* 2018). MYCN amplification and telomere maintenance are typical features (Ackermann *et al.* 2018). The first important resemblance between ALL and NB is polyploidy, which will be discussed in the next section. Next, the acquisition of telomere maintenance characterizing High Risk NB will be addressed, followed by the loss of entire chromosomes as observed in Hyperdiploid ALL and Low Risk NB. Finally, the role of complex SVs will be discussed.

3 Polyploidy

Almost all human cells are diploid, having two pairs of 23 chromosomes (2N). During the cell-cycle, the diploid state and DNA integrity needs to be maintained, for which the cell has evolved multiple checkpoints (see figure 1) (Davoli and Lange 2011). Cyclin dependent kinases (CDKs) and cyclines ensure that the cell-cycle phases alternate in the correct order, and replication is controlled such that it can only occur once every cell-cycle (Davoli and Lange 2011). Before karyokineses, the spindle assembly complex (SAC) ensures correct segregation of sister chromatids to maintain the diploid state (Davoli and Lange 2011). Finally, the cell-cycle is arrested upon DNA-damage and apoptosis will be induced if the damage cannot be repaired. This checkpoint is mainly controlled by p53, p16 and Rb (Davoli and Lange 2011).

Despite these checkpoints, physiological examples of polyploid cells exist that have multiple pairs of 23 chromosomes (for example 3N, 4N) (Davoli and Lange 2011). Placental trophoblast giant cells alternate between S- and G-phase to presumably accelerate growth (Gardner and Davies 1993)



Davoli T, de Lange T. 2011.
Annu Rev. Cell Dev. Biol. 27:585–610

Figure 1. Cell cycle in normal cells and the checkpoints that ensure genome integrity. In healthy cells, the cell cycle phases alternate in the same order from G1, S, G2 to M. During the G1 phase, the replication origins are licensed in order to be able to have DNA replication. Upon DNA damage, ATM and ATR can prevent the cell cycle from progressing to prevent errors persist. In the S-phase, DNA is replicated starting at the origins of replication. During the S-phase, Geminin inhibits the re-licensing to prevent overduplication. In the M-phase, the chromosomes are attached to centrosomes and segregated to form two daughter cells during cytokinesis. Karyokinesis is inhibited if the chromosomes are not properly attatched to prevent segregation erros. This image was made and published by Davoli and Lange 2011.

and hepatocytes have abortive mitosis, causing polyploidization (Celton-Morizur and Desdouets 2010). Although the benefits of polyploidy have not yet been elucidated, it has been proposed that it increases the metabolic capacity (Duncan *et al.* 2010). Polyploidy is also associated with different kinds of pediatric cancer, such as NB (Brodeur and Bagatell 2014) and central nervous system germ cell tumors (Satomi *et al.* 2022). Polyploidy allows cancer cells to more easily sustain acquired alterations and can lead to increased chromosome missegregation, due to an increased number of centrosomes. Polyploid cells can be formed through 1) cell fusion, 2) errors during the cell-cycle and 3) endoreduplication (Davoli and Lange 2011). Cell fusions are mainly caused by oncogenic viruses, although these have not been found for NB and ALL (Davoli and Lange 2011). Polyploidy is found in both Low and High Risk NB (Brodeur and Bagatell 2014). Low Risk NB presents with (near-)triploidy which is thought to be caused by whole genome duplication (WGD) and whole chromosome loss (Lundberg *et al.* 2013). In contrast, High Risk NB does not lose whole chromosomes after tetraploidization and is more frequently characterized by the acquisition of telomerase maintenance through *MYCN* amplification, TERT rearrangement or inactivation of

ATP-dependent helicase (Ackermann *et al.* 2018). Since this is the main difference between Low Risk NB and High Risk NB, telomeres are thought to play an important role in the tumorigenesis of High Risk NB (Peifer *et al.* 2015).

3.1 Telomeres

Telomeres are located at the ends of chromosomes and are important for maintaining chromosome integrity (Blackburn and Szostak 1984). During DNA replication, DNA-polymerase duplicates the DNA by using both DNA-strands as a template. DNA-polymerase cannot perform *de novo* DNA synthesis and thus requires an RNA-primer to which it adds nucleotides to the 3'-end. The RNA-primer is then removed and replaced by using the newly synthesized strand as a template (Ohki, Tsurimoto, and Ishikawa 2001). Consequently, towards both ends of the chromosome there is always a part of the 5'-end that cannot be replicated with at least the length of the RNA-primer; after removing the RNA-primer, there is no free 3'-end to extend. As a consequence, after each replication, the chromosome becomes shorter and DNA is lost. Without having a buffer, this would lead to the loss of genetic material. Chromosomes indeed have such a buffer, called telomeres, which consist of highly conserved tandem repeats (5'-TTAGGG-3') of double stranded DNA (dsDNA) and a single stranded DNA (ssDNA) overhang of the 3'-end (Turner, Vasu, and Griffin 2019).

Aside from preventing genetic loss during replication, telomeres serve another important purpose. Homology-directed repair (HDR) and nonhomologous end joining (NHEJ) are mechanisms that recognize and repair double stranded breaks (DSBs) in the DNA (McClintock 1941). This requires another mechanism that prevents DSB-repair at the chromosomal ends. To this end, telomeres are organised into a looped structure by self-invasion by the ssDNA overhang on its own dsDNA (Blackburn and Szostak 1984). Additionally, the telomeres are protected by shelterin complexes that stabilize the telomeres and prevent activation of HDR and NHEJ (Lange 2018). Thus, the telomeres prevent loss of genetic material during DNA replication and prevent DSB-repair.

3.2 Telomere Shortening

Even though shelterin complexes stabilize the telomeres, they also impose a disadvantage during replication. In addition to the inevitable shortening of chromosomes, shelterin causes replication fork stalling at the telomeric ends (Ohki and Ishikawa 2004), causing much more DNA loss than the length of an RNA-primer (Turner, Vasu, and Griffin 2019). Therefore, telomeres become shorter each cell division and eventually shelterin can no longer bind to the telomeres and the looped structure cannot be formed anymore (Turner, Vasu, and Griffin 2019). In healthy non-stem cells, this leads to senescence or apoptosis, preventing DNA loss possibly resulting in activation of oncogenes or deactivation of tumorsuppressor genes (Wright, Pereira-Smith, and Shay 1989). However, stem cells need to keep dividing much more, therefore needing to maintain their telomeres. Stem cells express telomerase reverse transcriptase (TERT), a protein capable of elongating the telomeres after replication. Elongation of the telomeres allows the stem cells to proliferate much longer than ordinary somatic cells (Hiyama and Hiyama 2007). Obtaining telomere maintenance is also one of the hallmarks of cancer, since it allows them to proliferate indefinitely (Hanahan 2022). This can be achieved through activation of TERT, the main method of telomere maintenance in cancer, or through the alternative lengthening of telomeres (ALT) pathway (Ackermann and Fischer 2019). In NB, TERT is activated due to amplification of the transcription factor neuroblastoma MYC oncogene (*MYCN*) and rearrangement of the *TERT* locus. Even though activation of TERT is not sufficient for malignant tumor progression (Ackermann and Fischer 2019), *MYCN* amplification is viewed as the main driver event of High Risk NB, since it is frequent in most High Risk NB tumors and is rarely acquired at a later stage (Bansal, Gupta, and Ding 2022). Telomere shortening could therefore be a possible driver event of High Risk NB, leading to *MYCN* amplification and ultimately

stabilization of the telomeres through the acquisition of telomere maintenance. There are two consequences of telomere shorteninig that can play a role in tumorigenesis: DNA-damage signalling and chromosome fusion.

3.3 DNA-damage signalling

Firstly, DNA-damage signalling can be induced by the loss of the looped structure (Turner, Vasu, and Griffin 2019), because linearization of the telomere can lead to activation of serine/threonine-protein kinase ATR (ATR) and serine-protein kinase ATM (ATM). These kinases, normally inhibited by shelterin-proteins, lead to the activation of multiple pathways, like p53, regulating the DNA-damage response resulting in senescence or apoptosis. Importantly, the most frequently recurring mutations in pediatric cancer occur in *TP53*, the gene encoding p53 (B. B. Campbell *et al.* 2021). *TP53* mutations are observed in Low Risk NB, High Risk NB and MLL-rearranged ALL, albeit with very low prevalence. It has been shown that prolonged DNA-damage signalling caused by telomere shortening in p53-deficient cells can induce tetraploidization through endoreduplication (Davoli, Denchi, and Lange 2010). This could explain polyploidization observed in (very) High Risk NB. However, compared to adult cancer, *TP53* mutations are still relatively rare in pediatric cancer (B. B. Campbell *et al.* 2021). It is striking that polyploidy is observed given the uncommon mutation of *TP53*. The role of *TP53* is therefore still unclear in pediatric cancer.

3.4 Chromosome fusion

Secondly, losing its looped structure makes the telomere indistinguishable from a DSB, inducing DSB-repair mechanisms like NHEJ and HDR. This can lead to the formation of dicentric chromosomes, fused chromosomes with two centromeres (Davoli and Lange 2011). When the fused chromosomes are attempted to be segregated in anaphase, they can form a chromatin bridge between the two daughter cells causing a lagging chromosome. This can lead to a failed cytokinesis, which can lead to a tetraploidization (Vitale *et al.* 2011). Not only the DNA is doubled twice when mitosis is omitted, but centrosomes as well. During the S-phase, centrosomes are duplicated in order two form two new cells. Tetraploidization therefore leads to a 4N cell with supernumerary centrosomes, which can lead to aneuploidy (Marthiens, Piel, and Basto 2012). It has been shown that chromatin bridge formation is present in NB cells indicating that chromosome fusion through telomere shortening could be important in the disease (Lundberg *et al.* 2013). In conclusion, this could indicate that telomere shortening causes chromosome fusion and is implicated in the tumorigenesis of NB.

4 Aneuploidy

As mentioned before, polyploid hepatocytes are physiological examples of polyploidal cells. It has been proposed by Duncan *et al.* 2010 that programmed polyploidization in hepatocytes yields genetic variability. Following polyploidization, the hepatocytes can lose chromosomes due to asymmetric mitosis, likely multipolar division caused by centrosome amplification (see figure 2A) (Duncan *et al.* 2010). This loss of chromosomes leads to an aberrant number of chromosomes that deviates from polyploidy resulting from WGD. Cells with such a karyotype are called aneuploid. Throughout literature, aneuploidy is referred to using a wide range of terminology, usually based on the closest multiple of 23. A karyotype with 45 chromosomes can then theoretically be named (high) hyperhaploid, near-diploid or hypodiploid. In ALL and NB we observe hyperdiploidy and near-triploidy and from here on hyperdiploidy will be used to prevent confusion. Hyperdiploid ALL is characterized by more than 50 chromosomes and has a good prognosis. Importantly, Low Risk NB is also characterized by hyperdiploidy (Salim *et al.* 2021) with good prognosis, with some cases even resulting in spontaneous regression and benign differentiation (Brodeur and Bagatell 2014). This could indicate that the mechanism leading to aneuploidy in Hyperdiploid ALL and Low Risk NB

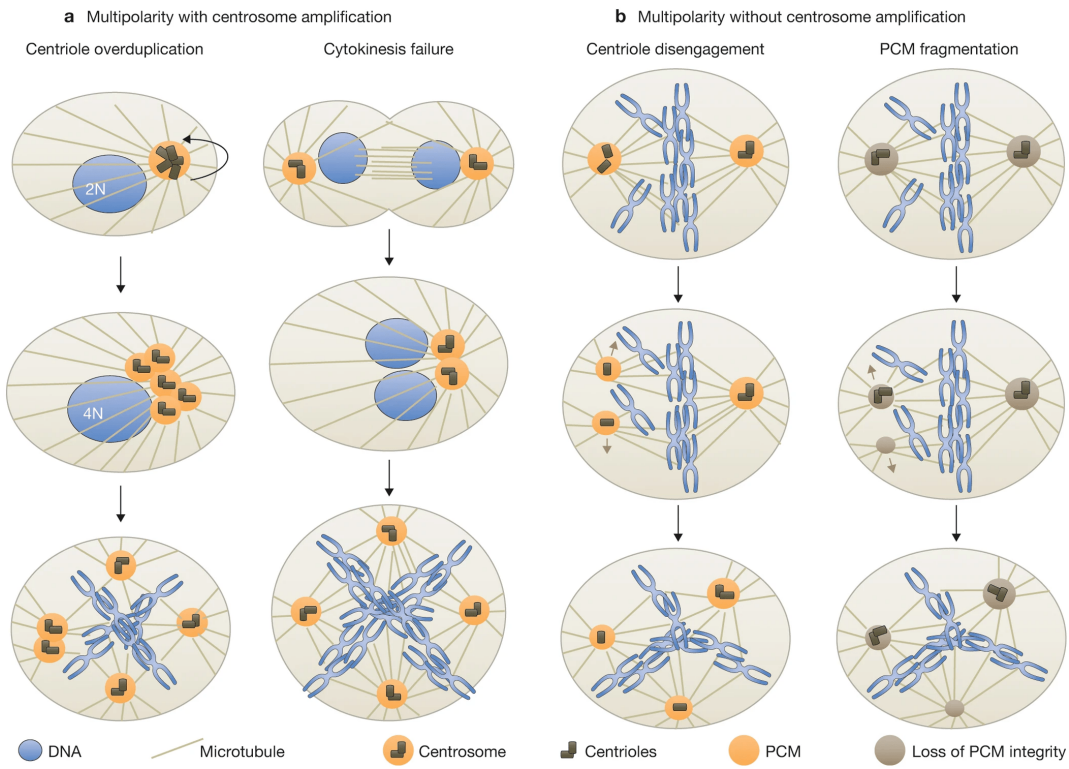


Figure 2. Multipolar divison with and without centrosome amplification. Multipolar division causes asymmetric segregation of chromosomes and can cause aneuploidy. **A)** Centrosomes can be amplified by two mechanisms. During the S-phase centrosomes are duplicated in addition to the DNA. Errors in the duplication of centrosomes can cause overduplication of the centrosomes. Additionaly, if cytokinesis fails, a tetraploid cell with supernumerary centrosomes is generated. This can also cause aneuploidy. **B)** Multipolarity can also occur without centrosome duplication. Centrioles that make up the centrosome can disengage causing multipolarity. Finally, the PCM can become fragmented, also resulting in multipolarity. This figure was made and published by Maiato and Logarinho 2014. Abbreviations: Pericentriolar Material (PCM), Diploid Nucleus (2N), Tetraploid Nucleus (4N).

could be the same. Although very little is known about the mechanism that causes aneuploidy in Low Risk NB, Hyperdiploid ALL has been studied more. Hyperdiploidy might be achieved through different mechanisms: through 1) formation of near-haploid cell followed by WGD; 2) chromosome loss after tetraploidization; 3) successively gaining chromosomes over multiple cell divisions; 4) a single irregular mitotic event (Paulsson *et al.* 2005). If the first mechanism has occurred then the resulting genomes would carry uniparental disomy (UPD) because the first step is formation of a near-haploid cell, thereby losing about half of the chromosomes. Paulsson *et al.* 2005 showed that this mechanism in very unlikely in the context of ALL given the lack of enough UPDs and in a subsequent publication they acknowledge the near-haploid lineage as very rare (Paulsson and Johansson 2009). Chromosomal loss after a tetraploidization event could not definitively be excluded because UPDs were present. Still, this mechanism was deemed unlikely for most cases. The allele dosages were analyzed to try to find evidence for the third and forth mechanism. The majority of cases showed equal allele dosage, indicating that one single mitotic event is most likely the cause of Hyperdiploid ALL (Paulsson *et al.* 2005). Multipolar division could be the underlying mechanism causing Hyperdiploid ALL.

In a normal division, the centrosomes move to two juxtapose positions in the cell, causing chromosomal segregation towards two poles. In an multipolar division, the chromosomes are pulled towards more than two locations, causing more than two daughter cells to be formed with an aberrant number of chromosomes (see figure 2) (Maiato and Logarinho 2014). Multipolar division

can occcur due to 1) centriole overduplication, 2) cytokinesis failure, followed by another complete cell-cycle, 3) centriole disengagement and 4) fragmentation of the pericentrioler material (Kalkan *et al.* 2022). It was shown that centrosome amplification is prevalent in B-cell precursor ALL (Guo *et al.* 2023), which could indicate that multipolar division could be the involved in Hyperdiploid ALL. Importantly, it was proposed that hyperdiploidy in Low Risk NB could also be caused by a single mitotic event (Gisselsson *et al.* 2007). However, upon examination of the intratumor diversity of chromosome numbers, it was deemed more likely that aneuploidy in Low Risk NB is caused by WGD and subsequent loss of whole chromosomes (Lundberg *et al.* 2013). This could indicate that Low Risk NB could be caused by WGD followed by multipolar division. Additionally, the loss of chromosomes in Low Risk NB might also be caused by lagging chromosomes. Therefore, aneuploidy in Low Risk NB and Hyperdiploid ALL might both be caused by multipolar division, though different mechanisms could also explain aneuploidy in Low Risk NB.

5 Structural Variation

In addition to driving WGD and aneuploidy, telomere shortening can lead to SV in the DNA (Yi and Ju 2018). SVs are large mutation with more than 50bp (Carvalho and Lupski 2016) and can be further divided into four basic types: 1) deletion, 2) inversion, 3) translocation and 4) duplication. With a deletion, a large portion of the chromosome is lost. In an inversion, the orientation of a part of the chromosome is inverted while the sequence remains intact within that inverted region. After a translocation, a region belonging to one chromosome has been attached to another chromosome. A duplication means that a large part of the genome is repeated more than in the reference genome. Despite the clear stratification of SVs, they are not independent. A combination of different SVs can be acquired as a consequence of a single catastrophic hit. A catastrophic hit is an event in which an error occurs during a physiological process. Different types of catastrophic hits have been found in cancer which result in a combination of SVs (Yi and Ju 2018).

Gene-fusions caused by the SV deletion have been found in both NB and ALL. This results in the activation of the oncogene FOXR1 in NB (Santo *et al.* 2012) and enhancer-hijacking in B-ALL (Yang *et al.* 2020).

5.1 Chromothripsis

Chromothripsis is an event of shattering a part of the chromosomes to pieces. After such an event, dozens of SVs can be observed. Most proposed mechanisms indicate that chromothripsis results from errors during mitosis which leads to shattering of the chromosome, followed by erratic repair by stitching DNA fragments back together. As a result, the broken fragments are joined with disregard to their original orientation and can be inverted and translocated. Some DNA fragments are not reincorporated in this repair and are thereby lost. Chromothripsis can be caused by two distinct mechanisms. The first mechanism involves generation of micro-nuclei and subsequent failures in DNA-replication. DNA-replication in micronuclei is impaired, presumably due to a lack of factors required for DNA-repair and -replication as a consequence of lower nuclear pore density (Liu *et al.* 2018). Incomplete DNA-replication in the micronuclei can then lead to shattering under mitotic signalling. The second mechanism is caused by fusion of two chromosomal arms due to telomere shortening (see section 3.4). In addition to tetraploidization, this event can also result in partial nuclear membrane rupture causing the chromatin bridge to be shattered by 3' repair exonuclease 1 (TREX1) activity. This mechanisms could explain why chromothripsis is often observed close to the telomeres (Yi and Ju 2018). The repair of the tens to hundreds of DSBs during chromothripsis results in a combination of deletions, inversions and reordering of the chromosome (Simovic and Ernst 2022). Such high reordering of the chromosome has not been found in ALL and Low Risk NB. In contrast, chromothripsis has been observed frequently in High Risk NB and is associated with poor

prognosis (Molenaar *et al.* 2012). In these tumors, chromothripsis might lead to rearrangement of the *TERT* locus. Additionally, chromothripsis has been found to lead to seismic amplification of *MYCN* (Rosswog *et al.* 2021).

5.2 Gene Amplification

During chromothripsis, some shattered fragments are not reincorporated into the chromosome during repair. These fragments can be lost or lead to the formation of genomic aberration in the form of extrachromosomal DNA (Yi and Ju 2018). One important catergory are the double-minute chromosomes, which are circular pieces of DNA without centromeres. Double-minute chromosomes are capable of self-replication and are an important cause of amplification in cancer (Thomas *et al.* 2004). After amplification, these double-minute chromosomes can be reincorporated into the chromosomes, which was identified in some High Risk NB tumors. This method of amplification was called seismic amplification (Rosswog *et al.* 2021). In addition to chromothripsis, the amplification step might also occur via the breakage-fusion-bridge (BFB)-cycle (Rosswog *et al.* 2021).

The BFB-cycle resembles the first step of chromothripsis. It involves the fusion of sister chromatids before anaphase due to telomere loss (see section 3.2). The fusion again leads to chromatin bridge formation, followed by an eventual chromosomal break. After breakage, the chromosomes in the daughter cells can fuse again and the cycle repeats. The difference between chromothripsis and the BFB-cycle is the result. Chromothripsis leads to a scrambling and rearranging of the chromosome, whereas the BFB-cycle can lead to gene amplification in one of the daughter cells and gene loss in the other (Yi and Ju 2018). A subgroup of ALL with very poor prognosis is a subgroup with intrachromosomal amplification of chromosome 21 (iAMP21) (Harrison 2015). Robinson *et al.* 2007 showed that iAMP21 could be caused by the BFB-cycle. However, in some cases iAMP21 was initiated by chromothripsis (Li *et al.* 2014). The similarity between chromothripsis and the BFB-cycle is the formation of dicentric chromosomes (Yi and Ju 2018). This could indicate that genomic amplification is caused by telomere shorteninig (Harrison 2015), but it could also be caused by a dicentric chromosome resulting from Robertsonian translocation (Li *et al.* 2014).

5.3 Chromoplexy

A balanced mutational event called chromoplexy was discovered in prostate cancer (Baca *et al.* 2013) and later in Ewing Sarcoma (Anderson *et al.* 2018). Chromoplexy can disable tumor suppressors through disruption and can activate oncogenes through fusion formation. Chromoplexy is a translocation between multiple chromosomes, commonly more than three without gain or loss of genetic material. It is thought that chromoplexy is caused by multiple coinciding double stranded breaks (DSBs) in different chromosomes, followed by DSB-repair that incorrectly matches the chromosomal fragments, though the exact mechanism has not yet been elucidated (Baca *et al.* 2013). It has been observed that chromoplexy occurs in chromosomal regions with active transcription (Baca *et al.* 2013). This could indicate there might be a common translational hub and that the DSBs and the repair happen in these translational hubs (Yi and Ju 2018). Although several gene-fusion have been found in ALL, no links have been made to chromoplexy. Instead, the gene-fusions are caused by translocations (Tomizawa *et al.* 2022; Kaczmarska *et al.* 2023).

6 Discussion

The mechanisms underlying pediatric cancer are poorly understood and need to be elucidated. To this end, similarities and differences between subgroups of ALL and NB have been compared to find possible overlap.

6.1 Telomeres Could Drive High Risk Neuroblastoma

High Risk NB is characterized by the acquisition of telomere maintenance and has a very poor prognosis. Because acquisition of telomere maintenance in itself is not sufficient for the development of a malignant tumor (Ackermann and Fischer 2019), it is highly unlikely that acquisition of telomere maintenance is an initiating event of High Risk NB. *MYCN* amplification, however, has been proposed to be the main driver event in the development of High Risk NB, which in turn can lead to the activation of telomere maintenance. This raises the question: what causes *MYCN* amplification in the first place? High Risk tumors also show tetraploidization, which can be caused by telomere shortening. Since telomere maintenance is likely acquired as a secondary step, this could indicate that telomere shortening drives *MYCN* amplification. It is being debated whether *MYCN* amplification could be caused by chromothripsis or the BFB-cycle and telomere shortening plays an important role in both of these catastrophic events. This further strengthens the hypothesis that telomere shortening could be a initiating factor to *MYCN* amplification in High Risk NB.

6.2 Seismic MYCN Amplification

Although chromothripsis can cause CNAs, it does not explain high level amplifications. However, it has been shown by Rosswog *et al.* 2021 that chromothripsis can cause the formation of extrachromosomal double minutes that can self-replicate, leading to oncogene amplification. This could tie the role of telomere shortening to amplification of *MYCN*. Therefore, I hypothesize that the *MYCN* amplified High Risk NB tumors are formed by initial shortening of telomeres, which leads to chromosome fusion followed by chromothripsis. When this event occurs on chromosome 2, this can then lead to *MYCN* amplification through self-replication of extrachromosomal double-minutes. Amplification of *MYCN* induces the acquisition of telomere maintenance, potentially restoring the chromosomal stability of the tumor. Under this hypothesis, I expect that cells that do not acquire telomere maintenance fast enough are not viable and do not lead to cancer development. Furthermore, some High Risk NB tumors present with *TERT* rearrangement (Peifer *et al.* 2015), which could potentially also be caused by telomere shortening induced chromothripsis. This could indicate that this proposed mechanism underlies High Risk NB.

Similarly to High Risk NB, gene amplification has also been observed in ALL. A subgroup of ALL is characterized by iAMP21 which appears to be caused by the BFB-cycle. Interestingly, in some cases iAMP21 appears to be initiated by chromothripsis. In these cases, there is a constitutional Robertsonian translocation, which results in a dicentric chromosome. This genomic aberration can then initiate the BFB-cycle (Li *et al.* 2014). This could indicate two possible mechanisms can lead to initiation of the BFB-cycle in ALL. Telomere shortening, like in High Risk NB appears to be the more common initiating step (Li *et al.* 2014) and chromosome fusion due to a Robertsonian translocation is a much more rare occurrence. Both result in the start of a BFB-cycle, which could indicate another similarity between NB and ALL. In conclusion, ALL and NB characterized by gene amplification are associated with poor outcome and can be caused by complex SV, either through the BFB-cycle or seismic amplification.

6.3 Hyperdiploidy in ALL and NB

In contrast to High Risk NB, the Low Risk subgroup lacks the acquisition of telomere maintenance and segmental chromosome losses are not typically observed. Instead, Low Risk tumors are characterized by whole chromosome gains and are often described as near-triploid. It is therefore unlikely that Low Risk NB is caused by telomere shortening. However, very little is known about the mechanism that drives this subgroup and research appears to be focused more on the High Risk group. Since the Low Risk group is approximately half of all NB cases (Ackermann *et al.* 2018), this could be explained by the fact that High Risk NB has a much worse prognosis, thereby gaining

researchers' attention, causing them to study the Low Risk group less. Importantly, the near-triploid karyotype of Low Risk NB strongly resembles Hyperdiploid ALL. This could indicate that the driving mechanisms behind Hyperdiploid ALL might be similar for Low Risk NB.

Currently, it is hypothesized that the majority of Hyperdiploid ALL is caused by a single mitotic event (Paulsson *et al.* 2005) and that hyperdiploidy via a tetraploid intermediate could be the cause in rare cases (Paulsson and Johansson 2009). Alternatively, a possible cause could be multipolar division, which has been found in another pediatric cancer, the Wilms tumors (Gisselsson *et al.* 2010). Centrosome amplification has been found in ALL cells (Guo *et al.* 2023), which could indicate that multipolar division indeed might be causing Hyperdiploid ALL. Multipolar division could also explain both single mitotic event hyperdiploid and hyperdiploidy via tetraploid intermediate, because multipolar division can be caused by several defects. Supernumerary centrosomes caused by a tetraploidization event could cause multipolar division. However, it is not yet clear how centrosome amplification is exactly involved in ALL. In agreement with Hyperdiploid ALL, it was proposed that Low Risk NB might be caused by a single event as well, like an asymmetric division (Gisselsson *et al.* 2007). However, more recently it has been proposed that Low Risk NB is caused by WGD followed by chromosomal loss. Furthermore, not all NB tumors exhibit multipolar mitosis (Lundberg *et al.* 2013). This further substatiates that two distinct mechanisms could be leading to aneuploidy in Low Risk NB and Hyperdiploid ALL. Either through 1) multipolar division caused by WGD or otherwise amplified centrosome or 2) WGD followed by lagging chromosomes (Lundberg *et al.* 2013). Therefore, it becomes very appealing to research the putative roles of multipolar division and lagging chromosomes in Low Risk NB and Hyperdiploid ALL.

6.4 Counting Chromosomes

Noticeably, in ALL-literature it is common to divide patients into subgroups by chromosome count (Haas and Borkhardt 2022). The reason behind counting the chromosomes is unclear to me. For example, cells with 47 chromosomes having two pairs of the normal chromosomes and one additional chromosome can be very different. First of all, trisomy of a small chromosome, like 21, would appear to have a different effect than trisomy of a large chromosome, like 1. Secondly, equal length chromosomes express wholly different genes and one would expect this to have a major influence on the cell. Furthermore, the term 'near-triploid' insinuates the cell resembles a diploid cell that has undergone WGD. However, this does not have to be the case at all, since there are many ways of becoming near-triploid. Moreover, Hyperdiploid ALL via a tetraploid intermediate is viewed as a rare subtype. Therefore, simply grouping patients by counting their chromosomes would seem to be a imperfect stratification method. Rather, the tumors should be distinguished based on the mechanisms that leads to their karyotypes. For example, Paulsson *et al.* 2005 distinguished Hyperdiploid ALL by examining UPDs. This divided the tumors into the rare hyperdiploid through tetraploidization and the more common hyperdiploid through a single event. Therefore, more research is needed to find common mechanisms that cause aneuploidy in Low Risk NB and Hyperdiploid ALL.

In order to more meaningfully partition ALL cases, it would be beneficial to better understand the mechanisms underlying the karyotypes. To know what sets aside a specific tumor can lead to more fitting personalized medicine. It therefore appears to be more meaningful to know what causes a certain number of chromosomes rather than how many chromosomes there are exactly. Furthermore, if tumor subgroups were to be based on their mechanisms of origin, this could lead to connections between different tumor types. For example, if Hyperdiploid ALL and Low Risk NB indeed share the same mechanism of becoming hyperdiploid, possibly their treatment could also be similar. Grouping tumors through their mechanisms of origin might potentially have an additional benefit. Since pediatric cancers are rare, a very limited amount of samples for research is available. It is therefore important to be able to increase the amount of knowledge that can be

obtained from those samples. If the mechanisms causing pediatric cancer are better understood and subgroups were to be based on this, possibly different types of tumor samples with the same mechanism of origin could be used in experiments. In conclusion, grouping tumors based on their mechanism of origin, rather than simple chromosome count could potentially lead to new insights in pediatric cancer and help find matches between existing treatment and other types of pediatric cancer.

6.5 Heterogeneity

Although Hyperdiploid ALL and Low Risk NB both are hyperdiploid, there is a striking difference in the chromosomes that are increased in numbers. ALL is characterized by a nonrandom gain of chromosomes X, 4, 6, 10, 14, 17, 18 and 21 and chromosomal stability (Paulsson and Johansson 2009). In contrast, both NB risk groups have been shown to have a high intratumor and intertumor chromosome number diversity (Lundberg *et al.* 2013). The intratumor diversity in particular might indicate that NB is more chromosomally unstable and advocates against a shared mechanism of hyperdiploidization for both cancer types given the stability of Hyperdiploid ALL (Paulsson and Johansson 2009). This might also explain why the emphesize on counting chromosome numbers found in ALL literature in absent for NB. A tumor with a highly diverse number of chromosomes cannot be meaningfully identified by its chromosome count, since it is highly diverse within the tumor. Given the heterogeneity of Low Risk NB, this could indicate that these tumors do not share a mechanism. However, as mentioned before, Hyperdiploid ALL has a rare occurrence of hyperdiploidy through tetraploidization. Therefore, both mechanisms could possibly cause aneuploidy in these tumors, though more frequently through tetraploidization in NB and through multipolar division in ALL. It is therefore important to identify whether Hyperdiploid ALL has subgroups that show intratumor heterogeneity like Low Risk NB. Finally, this could also indicate that a rare subgroup of NB is also caused by multipolar division, like Hyperdiploid ALL.

6.6 Fusion-Genes

A subgroup of ALL is characterized by having fusion genes. As discussed in section 5, fusions can occur through the complex SVs chromothripsis and chromoplexy. In both ALL and NB deletion-fusion have been identified and translocations are found in ALL. However, in most pediatric tumors there is an absence of complex SV, which contradicts the hypothesis that chromothripsis causes these fusion proteins. Furthermore, evidence suggesting chromoplexy causes these fusions is also lacking. The absence of these complex SVs could indicate that telomere shortening is not the underlying cause in pediatric ALL characterized by gene-fusions.

7 Conclusion

Hyperdiploid ALL and Low Risk NB have very similar karyotypes. They could possibly share a causal mechanism in multipolar division, similar to Wilms tumors, another pediatric cancer. However, given the intratumor heterogeneity of Low Risk NB, it would seem more likely that Low Risk NB is caused by WGD followed by chromosome loss due to lagging chromosomes. Telomeres do not appear to play an important role in these chromosomal number changes, in contrast to the High Risk subgroup of NB. I hypothesize that in High Risk NB, telomere shortening drives chromothripsis, which could lead to amplification of *MYCN* or rearrangement of *TERT*, both causing the acquisition of telomere maintenance, stabilizing the tumor and worsening prognosis. Similarly, acute lymphoblastic leukemia (ALL) characterized by iAMP21 could also be caused by telomere shortening, although it might also be initiated by Robertsonian translocation. In fusion-driven ALL, there is an absence of complex SVs, which could indicate that telomere shortening is not involved in the development of those cancer types. In conclusion, it seems that the similar karyotypes of ALL and

NB can be gained through different mechanisms, both mechanisms do not appear to be equally prevalent in both diseases.

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