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The prenatal origins of cancer

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Abstract

The concept that some childhood malignancies arise from postnatally persistent embryonal remnant or rest cells has a long history. Recent research has strengthened the links between driver mutations, and, embryonal and early postnatal development. This evidence, coupled with much greater detail on the cell of origin and the initial steps in embryonal cancer initiation, has identified important therapeutic targets and provided renewed interest in strategies for the early detection and prevention of childhood cancer.

Introduction

Human cancer is a multistep process, evolving over many years inexorably toward genomic instability and life-threatening cellular phenotypes. The recognition that a minority of cancer cells within a tumour possess potent embryonal features, or the plasticity to revert to embryonal features during postnatal life, has generated the cancer stem cell hypothesis[1], reflecting a tumour's almost limitless heterogeneity. Cancer stem cells are not replicates of their embryonal counterparts, but instead have some features of embryonal cells that provide a survival advantage suited to a particular time and place during tumour evolution[1]. By contrast, cancers presenting very early in a child's life often possesses embryonal features but must have moved quickly to genomic instability. This rapid evolution might occur when a cancer cell arises directly from an embryonal cell, or when a mature prenatal cell acquires embryonal properties which favour survival in the pre- and postnatal environment. Prenatal tumorigenic mutations might be more likely to occur in stem or progenitor-like cells that have characteristics, such as unrestrained self-renewal, that are essential in utero but fatal in postnatal life. Understanding the characteristics of these cells might well be important to understanding more about childhood cancer: why it develops, how best to treat it, and potentially how to prevent it.

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For some childhood cancers, such as retinoblastoma, infant acute lymphoblastic leukaemia (ALL) and malignant rhabdoid tumours, there is evidence of an embryonal cell of origin. However, it is unclear whether these embryonal cancers arise from embryonal cells *in utero* or as a consequence of a single oncogenic event in a more mature prenatal cell with rapid progression to genomic instability. The latter model is likely to be the case for mixed lineage leukaemia (*MLL*) fusion genes in infant ALL, and *INI1* mutations in malignant rhabdoid tumours[9, 10].

A unique characteristic of certain childhood cancers, such as neuroblastoma and Wilms tumour, is a precursor embryonal cell hyperplasia that presents in infancy but that has an overwhelming tendency to undergo spontaneous regression and cell death[4, 5]. More than 150 years ago pathologists first suggested that postnatally persistent embryonal remnant or 'rest cells' lay dormant in normal tissues but retained their capacity for growth and might later undergo malignant transformation[6–8]. Today, we know that during embryogenesis many more cells are produced than are required for organogenesis. Thus mechanisms, such as trophic factor withdrawal, are required for deleting cells in excess once organogenesis is complete. In rare instances, some embryonal cells can resist these cell death signals as a potential first pathological step toward cancer, later acquiring additional alterations which lead to postnatal malignant transformation.

Some types of cancer with embryonal histology, such as germ cell tumours, more commonly arise in early adulthood, however, it is unclear whether these malignancies represent cellular reversion from a mature to an embryonic form in adult life or postnatal persistence of embryonal cells, since there is no evidence of a rest disease pre-dating these cancers. Therefore, although the concept of rest cells is helpful in considering the developmental processes that might underlie cancer development in young children, lack of evidence for a rest disease before malignant transformation has been taken as evidence that few childhood cancers arise through this mechanism. However, a substantial number of childhood malignancies seem to arise prenatally. If all childhood malignancies with a suspected prenatal cell of origin are grouped together it is apparent that almost half of all childhood cancers might have prenatal origins (Table 1). In the case of B-lineage ALL (B-ALL), transient myeloproliferative disorder (TMD) and myeloid leukaemia-Down syndrome (ML-DS), driver mutations and causal gene rearrangements or balanced translocations are detectable in perinatal peripheral blood leukocytes years before clinical presentation, indicating these childhood cancers initiate *in utero*[2, 3].

Recent studies using genetically altered animal models, family linkage analyses, large scale expression profiling and genome-wide association studies (GWAS), have provided a rich vein of genes as possible drivers in prenatal cancers, many of which also have a role in embryonal development. This Perspective article focuses on four childhood malignancies with, we argue, the strongest evidence for a prenatal cell of origin: neuroblastoma, TMD and ML-DS, B-ALL and medulloblastoma. We discuss their prenatal origins in the context of normal tissue development and the potential cell of origin, as well as in the context of rest disease. Although rest cells are often seen as normal, we propose broadening the definition of rest disease to include any pre-malignant cellular hyperplasia detectable in the perinatal period. In this new definition, rest cells are already on the path towards malignancy, but

these cells retain the compulsion to die in the postnatal environment, as they still require additional postnatal changes for malignant transformation. The aim of suggesting this new definition is to facilitate discussion and experimentation aimed at identifying common aetiological features, strategies for early diagnosis and therapy, and, possibly prevention of childhood cancers of prenatal origin.

Neuroblastoma

Neuroblastoma is a malignancy of the sympathetic nervous system occurring almost exclusively in infancy and early childhood[11, 12]. Primary neuroblastoma arises in the adrenal medulla or sympathetic ganglia along the paravertebral axis. Considerable evidence suggests that neuroblastoma is initiated *in utero* during sympathoadrenal development and is a rest disease.

Candidate neuroblastoma initiation factors linked to sympathoadrenal development

The cells of the mature adrenal medulla and sympathetic ganglia originate in a transient collection of neural crest progenitors that rely on spatially and temporally regulated extrinsic signals for the later steps of migration, specification, divergence and maturation[13] (Figure 1). Sympathoadrenal cells from the neural crest in the trunk region of the embryo follow a ventral migratory pathway from the neural crest and neural tube, receiving signals from somites, ventral neural tube, notochord and dorsal aorta[13]. During normal sympathoadrenal development, expression of the proto-oncogene *MYCN* is high in the early post-migratory neural crest where it regulates the ventral migration and expansion of neural crest cells[14]. MYCN protein levels gradually reduce in differentiating sympathetic neurons[14, 15, 16] suggesting that sympathoadrenal maturation requires low or absent MYCN expression[14]. Consistent with this finding is the observation that *MYCN* transduction into quiescent rat sympathetic neurons reactivates cell cycling and blocks cell death induced by nerve growth factor (NGF) withdrawal[15,16].

After sympathoadrenal specification and expansion of the primary sympathetic ganglia, sympathoadrenal precursor development diverges into neuronal or chromaffin cell fates[13]. Based on expression patterns of differentiation markers, the earliest cell of origin for neuroblastoma is thought to be a neural crest cell specified to the sympathoadrenal lineage, which has not received or responded to cues that determine neuronal or chromaffin cell fate (Figure 1). Excess neural precursors undergo apoptotic cell death at the final stage of sympathoadrenal maturation[17], a process catalysed by local NGF deprivation. In zebrafish, persistent MYCN expression in sympathoadrenal precursor cells dramatically blocks development toward a chromaffin cell fate, leading to neuroblastoma[18]. Overexpression of human MYCN under the control of the rat tyrosine hydroxylase (*Th*) promoter in *Th-MYCN* transgenic mice is sufficient to recapitulate neuroblastoma rest disease (discussed in humans below) and tumorigenesis[15, 19, 20]. Transformation from a rest cell to a malignant neuroblast in *Th-MYCN* mice requires even higher levels of MYCN than are provided by the transgene. This occurs via transgene amplification and feed forward loops between MYCN and increased levels of NAD-dependent deacetylases, sirtuins 1 and 2, which increase MYCN stability[21, 22].

This cellular tolerance for high-level MYCN is surprising as supra-physiological levels of MYC expression should lead to apoptosis and senescence through the cyclin-dependent kinase inhibitor 2A, (CDKN2A) isoform 4 (known as ARF)–p53 stress response pathway[23]. Rest cell cultures from perinatal *Th-MYCN* mouse ganglia demonstrate lower basal and induced p53 levels than wild-type ganglia, which in turn are lower than p53 levels of mature ganglia[19]. When p53 is reactivated in rest cell cultures, the cells become sensitive to NGF deprivation[19]. Inactivation of the ARF–p53 pathway in neuroblasts can be accomplished by the Polycomb complex protein BMI1, acting as a p53 protein E3 ubiquitin ligase in *Th-MYCN* mice, or mutant anaplastic lymphoma kinase (ALK), through effects on PI3K and MAPK signalling in zebrafish[19, 24]. These findings indicate that there might be an inherent susceptibility to oncogenic stress in some embryonal cells lacking robust p53 stress responses.

These experiments mechanistically connect MYCN with the maintenance of neuroblast rests and as a driver of resistance to developmentally-timed trophic factor withdrawal signals at neuroblastoma initiation. The marked downregulation of high affinity nerve growth factor receptor (NTRKA) in *MYCN* amplified neuroblastoma cells blocks neural differentiation. Conversely, NTRKB responds to brain-derived neurotrophic factor (BDNF) which has survival- and growth-promoting actions on neurons. BDNF and NTRKB are expressed by MYCN-expressing neuroblastoma cells and maintain an autocrine cell survival loop that blocks differentiation and apoptosis[11].

Five weeks into human development, the ventrally migrating neural crest cell responds to bone morphogenetic protein signalling from the dorsal aorta to promote a transcriptional program that specifies the neuronal and catecholaminergic properties of the expanding primary sympathetic ganglia[13, 25, 26]. Murine achaete-scute homologue 1 (MASH1) then promotes paired-like homeobox 2A (PHOX2A) expression which, together with PHOX2B, drives the expression of enzymes for catecholamine biosynthesis. PHOX2B initiates a secondary transcriptional program, which controls terminal sympathoadrenal differentiation. Heterozygous germline mutations of *PHOX2B* are associated with a subset of familial neuroblastoma[27, 28]. Neuroblastoma driven by *PHOX2B* mutations often presents clinically in conjunction with the neural crest disorders Hirschsprung's disease and congenital hypoventilation syndrome, reinforcing the connection between neuroblastoma and defective neural crest development. PHOX2B mutant proteins appear to be oncogenic in a dominant negative manner and promote inappropriate proliferation in neural precursors[29, 30]. Interestingly, rest cells from *Th-MYCN* mice have high PHOX2B expression, indicating a possible indirect downstream link to MYCN[31]. Future experiments will uncover the finer detail of the regulatory relationships between these proteins in development and disease.

Paralogous RNA-binding proteins LIN28A and LIN28B regulate expression of the let-7 miRNA family during embryogenesis, controlling developmental timing and cell growth during neural crest cell lineage commitment[32]. Targeted expression of LIN28B to the developing neural crest of transgenic mice induces neuroblastoma[33]. LIN28B downregulates let-7 pre-miRNA's, which increases MYCN expression[33]. LIN28B is known to be crucial in the maintenance of stemness throughout embryonic development and this function might maintain the undifferentiated neuroblast phenotype[33, 34]. It will be important to determine

whether LIN28B transgenic mice recapitulate the rest disease seen in *Th-MYCN* mice. The known role of LIN28B in neurogenesis[35] fits well with a model of neuroblastoma initiation requiring aberrant regulation of developmental proteins. *LIN28B* can be amplified and highly expressed in some neuroblastoma tumours, which correlates with poor prognosis[33]. Interestingly, LIN28B–let 7 signalling through MYCN seems to be important in another putatively embryonal cancer, germ cell tumour[36].

ALK has a key role in early sympathoadrenal development to protect neuroblast growth in utero against nutrient deprivation[37, 38]. Germline and somatic mutations in *ALK* causing constitutive kinase activation are present in 8–10% of neuroblastoma cases and correlate with poor prognosis[39–42]. The most common and aggressive activating mutation of ALK, F1174L, is sufficient for tumour formation on neural crest-specific expression in transgenic mice[43] and on expression in neural crest cells transplanted into nude mice[44]. ALK-F1174L has been associated with *MYCN* amplification in human tumours and co-expression of ALK-F1174L and MYCN synergistically promotes tumour formation *in vivo* suggesting these may be co-operating events in tumour initiation[43]. Thus, aberrant MYCN expression or function in collaboration with disordered function of a small group of key neurodevelopmental regulators, are good candidates for neuroblastoma rest disease initiation. The exact order of these events, or the intrauterine factors which favour rest disease, are unknown.

Neuroblastoma as a sympathoadrenal rest disease

Several clinical and experimental features link neuroblastoma to defective embryogenesis and pathological rest disease. The embryonal origin of human neuroblastoma is supported by expression profiling showing that human foetal adrenal neuroblasts have gene signatures that are remarkably similar to neuroblastomas[45]. In some neuroblastoma patients new tumours form at different times and sites early in the child's life, suggesting a "field" of pre-malignant lesions, thus linking tumorigenesis to deregulated development.

A key feature of clinical or experimental rest disease is the compulsion to undergo cell death and spontaneous regression in the postnatal environment, thus mirroring features of embryonal cells *in utero* not required for organogenesis. The incidence of subclinical neuroblastoma and/or rests is much higher than the frequency of clinical disease. A small proportion of neuroblastoma patients present in early infancy with apparently metastatic tumour (stage 4S neuroblastoma) which undergoes regression without therapy. Autopsies of sympathoadrenal tissues from infants who have died of diagnoses other than cancer have shown an incidence of neuroblast rests 40-fold higher than the clinical disease[46]. Mass infant screening programs in the 1990s for the neuroblastoma tumour marker, urinary catecholamines, found subclinical tumours at a frequency more than 2-fold higher than the clinical incidence of neuroblastoma[47].

These mass screening programs aimed to prevent the later incidence of metastatic neuroblastoma by early tumour detection in infancy at the localised stage, followed by surgery, but were unsuccessful in this regard[47]. One explanation is that rest cells which later progress to metastatic neuroblastoma might already be fundamentally different at birth from the more common spontaneously regressing rest disease. This hypothesis is suggested by

work in *Th-MYCN* transgenic mice, showing a small number of perinatal rest cells, later selected for tumour formation, had undergone early *MYCN* transgene amplification[15]. Moreover, recent transgenic zebrafish modelling of neuroblastoma also indicates that there is a need for a co-operating signal, inactivating MYCN-induced apoptosis at tumour initiation[18]. Animal modelling confirms metastatic neuroblastoma can arise from rest cells[15].

The postnatal period of quiescent neuroblast rests in sympathoadrenal tissues offers a time window during early infancy when alternative forms of screening for rest disease might be envisaged based on a germline cancer susceptibility profile and recently described methods of detecting tumour DNA in peripheral blood[48]. Furthermore, it might be expected that drugs which restore the p53 stress response in rest cells, given briefly at this crucial early tumour phase might be effective in restoring the capacity for spontaneous regression and thus preventing malignant transformation.

Another prenatal cancer and potential rest-like disease that offers significant opportunities for early detection and therapy is TMD and ML-DS.

TMD and ML-DS

TMD is a megakaryocyte (MK) lineage disease that presents clinically at birth in 5–10% of children with DS, and has similarities to embryonal rest disease states[49, 50]. The clinical abnormalities associated with TMD resolve spontaneously in early infancy in almost all affected patients, however, approximately 20% of these children present 2–4 years later with ML-DS, an acute MK lineage leukaemia[49, 51].

MK development and TMD pathogenesis

TMD develops *in utero* during megakaryopoiesis. Megakaryocyte progenitors (MKPs) are first produced from haematopoietic stem cells (HSCs) in the yolk sac, aorto-gonad-mesonephros and thereafter the foetal liver[52–54]. Rarely, TMD can occur in infants without DS but all of these cases have trisomy 21 mosaicism in the marrow, supporting the key role of trisomy 21 in TMD pathogenesis. Moreover, the frequency of conversion to ML-DS is the same for patients with TMD linked to DS or non-DS trisomy 21 mosaicism[50, 52, 53, 55]. Foetal liver haematopoiesis in DS shows specific expansion of HSC and MKP compartments[57,58]. These trisomy 21-induced changes are likely to occur during definitive haematopoiesis from the yolk sac or aorto-gonad-mesonephros[57, 58]. Ts65Dn mice carry an extra chromosome with gene copies of most of mouse chromosome 16, which is orthologous to human chromosome 21[56]. Ts65Dn mice do not develop TMD spontaneously, but display abnormal megakaryopoiesis and chronic myelofibrosis[56].

MK development and differentiation is controlled by combinations of cytokines and other mediators present within a haematopoietic vascular and endosteal niche[58, 59]. Of the genes on chromosome 21, several are good candidates for leukaemogenesis: runt-related transcription factor 1 (*RUNX1*)*ERG*, *ETS2*, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A *(DYRK1* A) and GA binding protein transcription factor-α *(GABPA)*. RUNX1 expression is not increased in ML-DS[60] and trisomy of *Runx1* was not required for

the development of a myeloproliferative disorder in Ts65Dn mice[56]. The ETS transcription factor ERG, on the other hand, can immortalise haematopoietic progenitors[61, 62], and cooperate with a truncated version of GATA1 to generate a TMD-like defect *in vivo*[63].

RUNX1 interacts with additional factors, including the transcriptional activator GATA1[64]. GATA1 levels increase and GATA2 levels decrease during MK differentiation[59]. The *GATA1* gene is mutated in megakaryoblasts in all cases of TMD and ML-DS and these mutations, in exon 2 or at the exon 2–intron boundary of *GATA1*, lead to exclusive production of truncated GATA1, termed GATA1s[65] as early as 21 weeks of gestation[66]. GATA1s does not have an N-terminal transactivation domain, but retains both DNA-binding zinc fingers, and its expression leads to impairment of GATA1-mediated regulation of other transcription factors including GATA2, IKAROS family zinc finger 1 (IKZF1), MYB and MYC in foetal MKs[67]. Paired sample analysis by several groups indicates that the same *GATA1* mutation is present in TMD and matched ML-DS samples[53, 68–70]. However, neither the mutation site nor the nature of the mutation in *GATA1* exon 2 predicts progression to ML-DS[70] and surprisingly, Kanezaki *et al* found an association between low levels of GATA1s protein and a higher probability of progression to ML-DS[71].

TMD as a rest disease

TMD develops in utero when blood cell production resides in the foetal liver[52, 53, 72, 73]. Trisomy 21 creates MKP hyperplasia, and increased megakaryocyte early progenitor (MEP) clonogenicity, which precedes GATA1s expression[54, 57, 74]. Elegant mouse studies of the effects of a *GATA1s* knock-in allele showed that GATA1s expression leads to transient hyperproliferation of an embryonic MKP beginning in the yolk sac, then moving to the foetal liver, ultimately disappearing once haematopoiesis moves to the bone marrow postnatally[67]. Foetal liver-derived megakaryoblasts have been recently shown to differ from their adult counterparts by their low type 1 interferon production: type 1 interferons are known inhibitors of MKPs. This might help to explain why trisomy 21 and GATA1s have specific effects on foetal MKPs[75], and also indicates that exogenous interferon α might be an effective deletion therapy for residual foetal MKPs in TMD.

Thus, to date the evidence indicates that the progression from normal prenatal MKP to TMD requires trisomy 21 as the ‘first hit’[49] and then somatic *GATA1* mutation as the ‘second hit’. We think that it is likely that trisomy 21 and GATA1s combine to promote a perinatal TMD rest-like disease in some DS children by causing inappropriate MKP hyperplasia in the foetal liver. Like other rest diseases, MKP rest-like cells retain the compulsion to die in the perinatal period by as yet unknown mechanisms once haematopoiesis shifts to the bone marrow. At birth the TMD rest-like cells need a ‘third hit’ to sustain viability in the face of death signals. Whether these death signals also involve p53 is unknown. Transcription profiling studies of TMD and ML-DS samples indicate that driver mutations which activate WNT, JAK-STAT and MAPK–PI3K signalling are good candidates for a ‘third hit’[76] (Figure 2). Furthermore, recent TMD patient xenograft analyses demonstrate distinct subclones emerging on serial transplantation with genomic features of ML-DS, such as chromosome 16q deletion and chromosome 1q gain, which were present at low frequency in the initial TMD sample[77]. These observations are reminiscent of the process of clonal

selection for transgene amplification seen in *Th-MYCN* transgenic mouse neuroblast rests[15], and support the idea that even at birth rare leukaemogeneic or tumorigenic subclones exist among apparently regressing rest-like cell populations.

Collectively, we think that these data suggest that rest cells are not normal embryonal cells that have simply persisted postnatally. Instead postnatal rests, like cancer stem cells, have retained embryonal features at the early stages of a traditional pathway towards tumorigenesis. Recent studies using next generation sequencing of peripheral blood DNA from DS neonates to analyse for GATA1 mutations indicates the incidence of TMD may be in the range of 30%[78]. These findings suggest a strategy of predictive testing for TMD in DS neonates and pave the way for therapeutic intervention studies for TMD rest-like disease as a method of leukaemia prevention.

Another childhood cancer where leukaemia marker genes have been identified in the perinatal period suggesting prenatal origins is B-ALL. However, the absence of a clinical B-ALL rest disease and the rarity of the premalignant clone in the perinatal period means that application of preventative disease strategies for B-ALL will be more difficult.

B-ALL

ALL is the most common childhood malignancy, and arises in B- or T-lineage lymphocyte progenitor cells. We confine our discussion to the more common B-lineage ALL and B cell development, since T-ALL generally presents in adolescence, and most of the evidence for a prenatal origin has been obtained using samples from children with B-ALL.

Leukaemogenesis begins prenatally in children with B-ALL

Leukaemic cells from identical twin pairs with concordant B-ALL share unique, clonal, non-constitutive chromosome rearrangements, even when diagnosed years apart, indicating that leukaemogenic molecular alterations occur *in utero*[79, 80]. Further support for the idea of a prenatal origin came from a study of 5-year-old monozygotic twins aimed at identifying leukaemogenic markers[81]. These patients presented with B-ALL and concordant translocation of *ETS* variant 6 and *RUNX1* genes (also known as *TEL-AML1*). The leukaemic cells also showed distinct immunoglobulin heavy-chain (*IgH*) and immunoglobulin kappa deleting element (*IgK-Kde*) gene rearrangements in neonatal blood spot DNA, indicating that separate preleukaemic clones must have evolved before birth[81]. Using *IgH* rearrangement as a marker for leukaemic clones, several groups have now shown that pre-leukaemic cells can be detected in the neonatal blood spot of the majority of childhood B-ALL patients, indicating that the leukaemogenic process begins *in utero*[2, 82] (Figure 3).

The *TEL-AML1* fusion gene is a clonal feature of B-ALL cells at diagnosis in more than 20% of patients, but is present on neonatal blood spot analysis at a much higher frequency than the incidence of B-ALL in childhood[83–85]. Studies of a monochorionic *TEL-AML1*-positive twin pair (one preleukaemic and one leukaemic), have established that the presence of *TEL-AML1* in B cells is sufficient to generate a population of pre-leukaemic cells and act as a ‘first hit’ mutation that results in altered self-renewal and survival properties[86].

However, low level *TEL-AML1* expression only is tolerated by pro-B cells since these cells terminally differentiate in the long term[87]. Although *TEL-AML1* has been found in peripheral blood leukocyte DNA from healthy adults (0.5–8.8%), it is much more frequent in young adults[83], presumably representing pre-leukaemic clones which originated prenatally. *TEL-AML1* expression is, of course, not part of the normal B cell developmental program. Thus, TEL-AML1 operates as a weak oncogenic event in prenatal HSCs or precursor B-cells to generate a pre-leukaemic state which, like precancerous neuroblasts and megakaryoblasts, have a short lifespan in the postnatal environment. *TEL-AML1* may function as an oncogene only in neonates owing to specific characteristics of foetal B cells (Box 1), thus linking TEL-AML1-initiated leukaemia to the embryonal environment. Overexpression of the erythropoietin receptor, early B-cell factor 1 (*EBF1*), and deregulated transforming growth factor β responses, might have a role in survival of *TEL-AML1*-initiated pre-leukaemic cells[88–90]. Both the breakpoint cluster region (*BCR*)-*ABL1* and mixed lineage leukaemia (*MLL*)-eleven nineteen leukaemia (*ENL)* fusion genes can occur prenatally and might initiate B-ALL[91, 92]. However, the pre-leukaemic clone also remains clinically silent in the absence of additional changes, such as an *IKZF1* mutation[91].

We hypothesise that B-ALL may also follow a traditional tumorigenic pathway which begins in prenatal, partially transformed cells which have adopted characteristics favoured by the embryonal, but not the postnatal, environment. Consistent with our proposed broader definition of rest diseases, B-ALL may also be preceded by clonal hyperplasia of a small transient B cell population which, like neuroblast rests and TMD rest-like cells are destined to die unless further changes accelerate the path toward malignancy. More detailed analysis of genetically modified animal models of B-ALL for rest hyperplasia, and its spontaneous regression, would strengthen the hypothesis that B-ALL begins as a rest disease. In this regard, the recent description of mutant p53 as another familial B-ALL predisposition gene suggests the p53 stress response may be defective in B-ALL rests, much like neuroblasts from the *Th-MYCN* mice[15, 19, 93, 94]. However, for B-ALL to be considered as a rest disease, several important facts need to be established: the exact embryonal timing and tissue of initiation, and the leukaemogenic order and relative inter-dependency of the candidate initiator mutations with B cell homeostasis in the prenatal environment. The susceptibility of foetal B cells to the weak TEL-AML1 oncoprotein might be due to the different cell intrinsic responses of HSCs or innate B-1 precursor cells, or cell extrinsic susceptibilities of the foetal liver compared with the postnatal bone marrow (Box 1).

More evidence for rest-like disease in another embryonal cancer, medulloblastoma, exists in mouse models. However, like B-ALL there is no clinical presentation of a rest-like disease in patients who develop this brain tumour.

Medulloblastoma

Medulloblastoma arises from the developing cerebellum and adjoining structures, and is the most common malignant brain tumour of childhood. It represents four distinct disease entities: the sonic hedgehog (SHH) and wingless-related integration site (WNT) subgroups are named based on the predominant signalling pathway driving tumorigenesis, whereas

group 3 and group 4 medulloblastoma exhibit a more pleiotropic aetiology and do not have an easily identifiable oncogenic driver pathway (Figure 4).

Cerebellar development and medulloblastoma tumorigenesis

The fully developed cerebellum comprises an outer cell-sparse cortical layer (molecular layer), a Purkinje cell layer (PCL), and an internal granular layer (IGL), the latter is comprised of cerebellar granule cells (GCs) that form excitatory connections with Purkinje neurons[95]. Developmentally, the cerebellar anlage arises from the dorsal part of the anterior hindbrain, delineated by expression of the homeobox proteins orthodenticle homologue 2 (*OTX2*) anteriorly and *HOXA2* posteriorly[96, 97]. *OTX2* is a candidate driver for some group 3 medulloblastomas as it is amplified in 20% of these tumours, and is frequently overexpressed in SHH-independent subgroups[98]. Expression of OTX2 is abundant in the developing brain and silenced in the adult brain. OTX2 has recently been shown to repress differentiation in medulloblastoma cells, suggesting that amplification of *OTX2* could generate a rest-like disease[99]. The observation that OTX2 also drives proliferation and can upregulate MYC, suggests that OTX2 might also be important in transforming rest-like cells into medulloblastoma[98, 100].

The first germinal centre of the developing cerebellum initiates along the fourth ventricle in the dorsomedial ventricular zone (VZ), and gives rise to Purkinje cells and several other types of cerebellar interneurons[101]. Neural tube closure forms the rhombic lip, the anterior portion of which provides a second germinal zone of rapidly proliferating cells that express radial glial markers and gives rise to protein atonal homologue 1 (or MATH1)-positive granule cell progenitors (GCPs)[102, 103]. From 24 to 40 weeks gestation in humans, the volume of the developing cerebellum enlarges 5-fold, corresponding to more than a 30-fold increase in cerebellar cortex surface area[104, 105]. This expansion is largely imparted by the rapid proliferation of GCPs that give rise to cerebellar GCs and almost all cerebellar cortical neurons[101, 106]. Proliferation of these neuronal progenitors depends on SHH produced by Purkinje neurons: inhibiting SHH signalling blocks the proliferation and migration of GCPs[107]. GCPs migrate rostrally along the surface of the developing cerebellum to form the external granule layer (EGL), where they continue to divide and eventually migrate inward to form the IGL, with consequent depletion of the EGL[108, 109]. This process of GCP maturation and concurrent spontaneous cell death of excess GCPs is complete by the postnatal age of 20 months in children[110].

The connection between SHH signalling and medulloblastoma was initially made in the context of Gorlin syndrome, an autosomal dominant disorder that causes developmental defects and predisposes individuals to several cancers, including medulloblastoma[111]. Fine mapping revealed the putative gene to be highly homologous to the Drosophila gene *Patched* (*PTCH1*)[112]. Loss of chromosome 9q, which contains *PTCH1*, occurs in ~30% of all SHH tumours[113]. The PTCH1 protein is an essential negative regulator of SHH signalling, thus mice heterozygous for *Ptch1* develop cerebellar medulloblastoma[114]. Other germline mutations that predispose children to SHH medulloblastoma occur due to loss-of-function mutation in Suppressor of fused homolog (*SUFU)*, a negative regulator of the SHH signalling[115]. Amplification of GLI family zinc finger 2 (*GLI2*), a positive regulator of the

SHH signal, is also implicated in medulloblastoma development[116]. In a mouse model of SHH-induced medulloblastoma, tumour formation was preceded by GCP hyperplasia in the first week of life which regressed before later emerging as a malignant tumour[117], in a manner similar to neuroblast rests and neuroblastoma in the *Th-MYCN* mice[15]. However, in vivo labelling studies following a rest cell to tumour transformation have not yet been performed in either model. *In vitro*, the murine GCPs were resistant to SHH withdrawal and this feature was partially MYCN-dependent[117]. Neural stem cells (NSCs) and GCPs have repeatedly been shown to be the cell of origin for SHH medulloblastoma[118–122].

MYCN and its homologue *MYC* have crucial roles in all medulloblastoma subgroups. Brain-specific germline deletion of mouse *Mycn* results in cerebellar dysplasia[123]. Expression of MYCN is essential for SHH medulloblastoma in mouse models[124] and amplification of *MYCN* marks a subset of poor-outcome SHH-driven human tumours[125]. Moreover, tumorigenic SHH signaling markedly increases MYCN expression in GCP cells due to effects on MYCN protein stability[117]. MYCN is also expressed in WNT and group 4 tumours, with targeted expression of MYCN or MYC driving SHH-independent medulloblastoma in transgenic mice[126–128]. Group 3 tumours express MYC and have amplification of *MYC* rather than *MYCN*[129]. Thus, there are many similarities between the role of MYCN in generating neuroblast rests and GCP hyperplasia in neuroblastoma and medulloblastoma, respectively.

WNT signalling has many roles in neural development and aberrant WNT signalling in NSCs of the cerebellar ventricular zone induces transient proliferation and impaired differentiation[130]. Individuals with Familiar Adenomatous Polyposis (FAP) and WNT pathway-driven colorectal cancer also have an increased risk of developing medulloblastoma[131]. Mutations that increase WNT signalling in members of the WNT signalling pathway have been described in sporadic medulloblastomas[132–134]. Recent data from the Medulloblastoma Advanced Genomics International Consortium showed that β-catenin (*CTNNB1*) displayed canonical exon 3 deletion in the vast majority (70–80%) of WNT-subgroup medulloblastoma[116, 133, 135].

Insight into the cell of origin for WNT medulloblastomas comes from two mouse models, both involving the conditional expression of degradation-resistant β-catenin under the control of fatty acid binding protein 7 (*Fabp7*; also known as *Blbp*) in the context of *Trp53* deletion (*Blbp-cre:Ctnnb1:Trp53*). The addition of MYC expression increased penetrance from 4% ($Trp53^{+/-}$) and 15% ($Trp53^{-/-}$) to 83%, suggesting that activation of β-catenin and WNT signalling alone are only weak oncogenic events[136, 137]. These MYC-driven medulloblastomas were evident in young mice and were formed from $BLBP^{+}$, $OLIG3^{+}$ mossy-fibre neuron precursors that are present in the brainstem between E11.5 and E15.5, consistent with the radiological observation that WNT medulloblastomas are frequently located within the fourth ventricle and infiltrate the dorsal surface of the brainstem[126, 137, 138]. This observation was consistent with the model of $OLIG3^{+}$ neural precursors representing a brain stem rest disease preceding WNT medulloblastoma.

The pathogenesis of group 3 and group 4 medulloblastoma is less clear, yet they account for over 60% of all medulloblastoma cases[139]. Targeted inducible expression of MYCN to the

postnatal cerebellum using the glial high affinity glutamate transporter (*Glt1*) promoter (*Glt1-tTA:TRE-MYCN-Luc*, or GTML mice) leads to tumours with a transcriptional profile of group 3 medulloblastoma[126]. GTML mice had a normal EGL, suggesting that group 3 medulloblastoma could originate from a cellular population that is distinct from SHH medulloblastoma. However, proliferating GCPs isolated from cyclin-dependent kinase inhibitor 2C (*Cdkn2c*)$^{-/-}$, *Trp53*$^{-/-}$, *Math1-GFP* mice generate group 3 medulloblastoma when transformed with MYC, but not MYCN[127]. Furthermore, group 3 tumours have been modelled through MYC transformation of postnatal cerebellar stem cells marked by the expression of prominin 1 (also known as CD133) and lack of neuronal or glial markers[128]. Thus, it appears that a single oncogene can give rise to multiple different medulloblastoma tumour subtypes, depending on the developmental age and anatomical origin of the transformed cell, indicating that susceptibility to medulloblastoma is highly dependent on embryonal site and stage[140].

Unifying features of cancers with a prenatal origin

The work of Bishop and Varmus defined the forces driving human cancer as aberrations of our normal adult self [141, 142]. Embryonal cancer initiation appears to be an aberration of our prenatal self. We propose here a new definition of rest cells which includes all cancers with a prenatal origin whether they arise directly from an embryonal cell, or a more mature prenatal cell which has acquired pathological properties that favour survival in the postnatal environment. Using this new definition of rests, neuroblastoma and TMD appears to initiate prenatally in cells which display resistance to cell death, numerical excess, and differentiation arrest at a stage of tissue ontogeny several steps distal to the earliest progenitor cell for each organ (Figure 5). We hypothesise that B-ALL cells might also pass through this phase during evolution toward the frankly malignant state. Mouse models suggest similar features exist for some medulloblastoma subtypes. Indeed, much of the support for rest-like disease in TMD, B-ALL and medulloblastoma, comes from mouse models. Thus, these findings raise interesting questions, but do not indicate definitively that the same pathways operate in humans. We propose that rests or rest-like cells are groups of abnormal embryonal cells which have undergone changes that allow them to persist through cell deletion signals in the postnatal environment. Yet they retain the capacity for self-destruction in the postnatal milieu unless other tumorigenic factors intervene to cause genomic instability, a feature which may offer an opportunity for rest deletion therapy. Neuroblastoma and TMD modelling indicates that all rest cells are not equal at birth, with only a small number undergoing transformation after later exposure to unidentified postnatal environmental influences[15, 77]. The properties that permit some rest cells to remain dormant after birth when other adjacent rest cells are dying is currently unknown.

For neuroblastoma and medulloblastoma, the timing and location of the rest-like disease *in utero* coincides with a point in the tissue-specific maturation pathway when rapid cell expansion is required, quickly followed by an orderly transition to a terminally differentiated state. Substantial cell death must occur during the final shaping of the nervous system. It is resistance to these death cues which must accompany an inappropriate replication stimulus, such as continued or high-level MYCN expression. We postulate that individual variation in factors required to: negatively regulate MYCN expression in specific tissues during

embryogenesis, activate the apoptosis or senescence response to aberrant MYCN expression, or activate death responses imposed by trophic factor withdrawal, may explain individual susceptibility to rest disease.

From early in embryogenesis, haematopoietic precursors are in a continual state of replication and self-renewal, in contrast to terminally differentiated sympathetic ganglia and cerebellar neurons. Thus, while both TMD and B-ALL may not arise directly from an embryonal cell, the initial steps in each pathway toward postnatal malignancy involve progenitor cells acquiring characteristics that are favoured in the prenatal development. For TMD, and possibly B-ALL, the timing of rest initiation coincides with a unique predominance of foetal liver haematopoiesis. In the case of TMD, low interferon responses may be permissive for the survival of trisomy 21-primed and *GATA1s* mutant MKPs beyond the perinatal period in the non-permissive environment of the bone marrow [75].

The rest-to-cancer cell transition occurs in early childhood indicating that some rest cells must have the capacity for rapid progression toward genomic instability. One explanation for the speed of this process is inherited germline loss-of-function mutation in one allele of a tumour suppressor gene[143]. Another recently proposed mechanism is chromothripsis, whereby a single catastrophic event can lead to massive genomic rearrangement affecting more than one chromosome, rather than the incremental acquisition of single oncogenic mutations over decades[144–146]. An additional factor which may accelerate progression from rest to cancer are several recently described feed-forward loops, which increase the expression of MYCN to very high levels through the effects of SIRT1, SIRT2 and aurora kinase A on MYCN protein stability[21, 22, 147]. We propose that additional factors favouring rapid malignant transformation of rest cells are those properties of growth and self-renewal that are inherent in some embryonal cells.

Although chemotherapy, surgery and radiotherapy have improved the cure rates for childhood cancer, this has come at a significant cost in terms of the severe acute and long-term side-effects of therapy, and, considerable consumption of the paediatric health economy. Early evidence from screening trials in children at increased risk of cancer development due to germline p53 mutations has revived interest in strategies for child cancer prevention[148, 149]. While early detection of neuroblastoma using increased urine catecholamines failed to detect all neuroblastoma, one can envisage greater success when genomic susceptibility markers are incorporated into predictive algorithms for postnatal rest disease. Rest deletion therapy aimed at transiently restoring p53-mediated or interferon-mediated cell death responses or blocking MYCN, ALK, PI3K or MAPK signals in rest cells is an exciting strategy for childhood cancer prevention, which must first be demonstrated to be effective in mouse models of embryonal cancer. It is apparent that, with current models and a clearer understanding of the prenatal mis-steps that initiate rest disease, these novel strategies can be contemplated for the first time.

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Glossary

Neural crest	is a transient collection of multipotent embryonic progenitors in the developing ectoderm that gives rise to a multitude of different cell types including melanocytes, craniofacial chondrocytes and osteocytes, smooth muscle myocytes and peripheral nervous system neurons
Somites	are bilaterally paired blocks of mesoderm that form along the anterior-posterior axis of the developing embryo
Anlage	the initial clustering of embryonic cells from which an organ, or part of an organ, will form
Rostrally	situated toward the oral or nasal region, or in the case of the brain, toward the tip of the frontal lobe
Thrombocytopaenia	a platelet number below the normal range, in the peripheral blood
Neonatal blood spots	cards with drops of blood collected from newborn baby's heel, which are used for screening neonates for rare but serious metabolic conditions
Innate B-1 cells	B-1 cells with innate sensing and responding properties
Marginal zone B cells	B cells from the marginal zone of the spleen, a unique lymphoid area located at the interface between the circulation and the immune system
Adaptive B-2 B cells	B-2 cells responsible for the production of antibodies during adaptive immunity
Chromothripsis	process whereby a single catastrophic event within the genome leads to multiple genetic alterations across one or more chromosomes

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Box 1 Regulation of B-cell development and B-ALL leukaemogenesis

Haemogenic endothelium from the mouse E9 yolk sac and the para-aortic splanchnopleura independently give rise to the first B progenitor cells that preferentially differentiate into innate B-1 cells and marginal zone B cells, but not into adaptive B-2 cells[150]. Thereafter, B cells are generated from haematopoietic stem cells (HSCs) in the foetal liver until shifting to the bone marrow from birth onwards. Foetal liver-derived B cells exhibit some descriptive and functional differences from bone marrow-derived B cells, such as low terminal deoxynucleotidyl transferase expression, and a restricted diversity of the IgH variable and hinge region repertoire[151]. HSCs isolated from foetal liver can differentiate into B-1 and B-2 cells, however, B-1 production predominates until the bone marrow takes over and the B-2 postnatal program becomes established[152]. In embryonal or adult haematopoietic tissues, HSCs differentiate into lymphoid-primed multipotent progenitors (LMPPs) which express high levels of fms-related tyrosine kinase 3 (FLT3), lose megakaryocyte and erythrocyte progenitor (MEP) potential, and retain the capacity to differentiate into lymphoid and myeloid cells[153]. LMPPs can differentiate into early lymphoid progenitors, the precursors of bone marrow-derived common lymphoid progenitors (CLPs), which give rise to pre-pro-B cells expressing B cell lineage-associated genes[154, 155]. Mice deficient in FLT3 ligand have reduced CLP levels, whereas mice deficient in *Flt3* and interleukin-7 receptor (*Il7r*) do not develop B cells[156, 157].

A plethora of studies has revealed structural rearrangements, deletions, amplifications and point mutations in genes encoding regulators of B lymphocyte development in B-ALL patient samples, many of which are candidates for leukaemia initiation. Many are transcription factors that regulate B-cell development[158]. *IKZF1*-deficient HSCs exhibit reduced expression of IL-7R and FLT3 and loss of LMPPs, while reduced expression of IKZF1 in mice results in impaired transition from the pro-B cells to pre-B cells. *Ikzf1−/−* mice completely lack B cells[159–161]. *IKZF1* is deleted in most BCR-ABL1-positive B-ALL cells and mice expressing mutant IKZF1 develop ALL[162].

Conditional deletion of *Pax5* in mature B cells leads to de-differentiation toward uncommitted HSCs[163]. Somatic deletion, mutation or rearrangement of *PAX5* is detectable in more than 30% of children with B-ALL[164]. *PAX5* DNA-binding and internal deletion mutants act as hypomorphic alleles with weak competitive activity[164]. Hypomorphic *PAX5* mutations have recently been described in studies of two affected families with inherited B-ALL, thus representing familial B-ALL predisposition genes[165].

E2A, which encodes the E protein transcriptional regulators E12 and E47, activates expression of forkhead box O1 (FOXO1), and is required for CLP formation and B lineage specification[166–168]. EBF1 is necessary for pre-pro-B cells to transit to the pro-B stage[169]. Forced overexpression of EBF1 in both wild type and *E2A*-deficient HSCs leads to B cell differentiation[170].

RUNX1 is indispensable for generating CLPs[171], and, the expression of PAX5, E12, E47 and EBF1 is reduced in B cell precursors lacking RUNX1[170]. *Runx1* deficiency in mice leads to a severe reduction in CLPs, and a myeloproliferative phenotype[171].

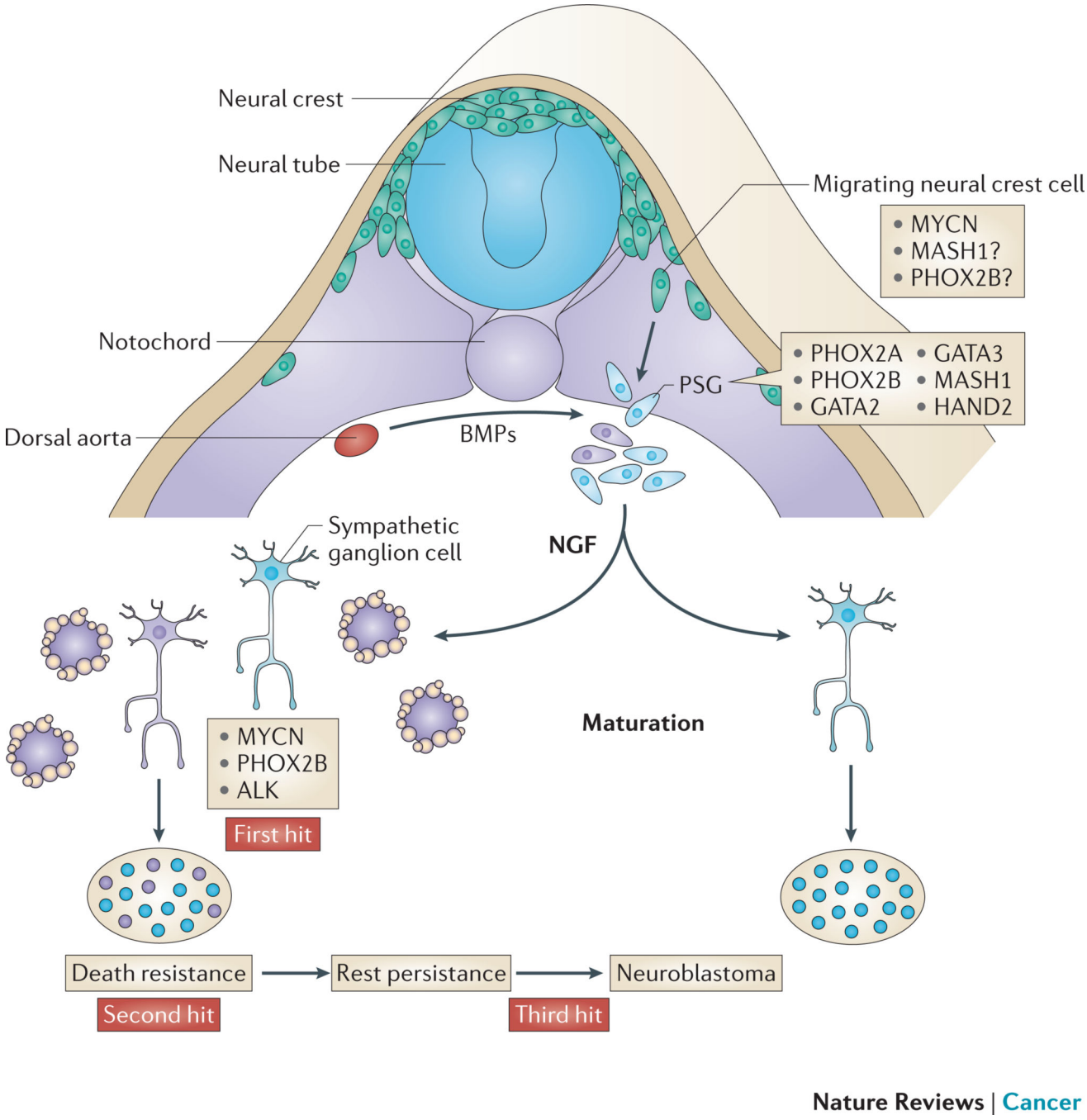

Figure 1. Neural crest development and neuroblastoma

Neuroblast progenitors migrate from the neural crest (nc), around the neural tube (nt) and somites (so) to a region immediately lateral to the notochord (no) and the dorsal aorta (da) under the influence of MYCN and bone morphogenetic proteins (BMPs). At this site the cells undergo specification as the primary sympathetic ganglia (psg) before divergence into neural cells of the mature sympathetic ganglia (sg) or chromaffin cells of the adrenal medulla (am). MYCN is a first hit by virtue of the observations from the tyrosine hydroxylase (*Th*)-*MYCN* transgenic mouse model, whereas mutations/alterations in

anaplastic lymphoma kinase (*ALK*) and paired-like homeobox 2 (*PHOX2B*) are inherited human susceptibility genes. Local access to nerve growth factor (NGF) determines whether the normal sg matures into a terminal ganglion cell or undergoes apoptotic cell death. A relatively common pathologic state is postnatal survival of neuroblast rest disease which requires the cell destined to become malignant to be resistant to trophic factor withdrawal before these persistent rest cells undergo a third change to induce transformation, which presents as a clinical malignancy in early childhood. MASH1, murine achaete-scute homolog 1; HAND2, heart and neural crest derivatives expressed 2; NF-M, neurofilament medium polypeptide.

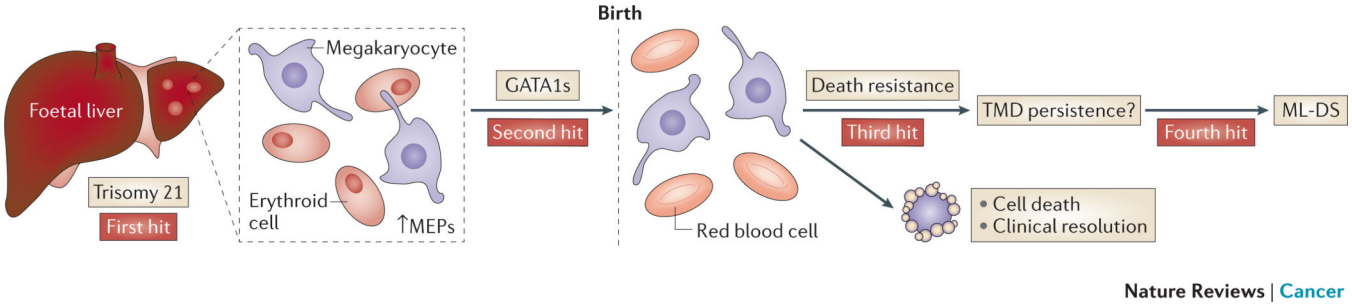

Figure 2. TMD and ML-DS

Children with Down Syndrome (DS) can develop Transient Myeloproliferative Disease (TMD) at birth which can later transform to Myeloid Leukaemia (ML-DS). The production of megakaryocyte-erythroid progenitors (MEPs) is enhanced in the foetal liver of children with DS and trisomy 21 as a first hit. Some megakaryocyte precursor cells develop a second hit mutation in *GATA1* resulting in a mutant, truncated protein, GATA1s. All children with TMD and ML-DS exhibit GATA1s and more than two copies of chromosome 21. TMD resolves clinically in almost all patients to later present as ML-DS in 25% of TMD cases. Thus, some TMD cells at birth must survive through unique death resistance mechanisms and may undergo further changes which lead to genomic instability and clinical presentation as ML-DS.

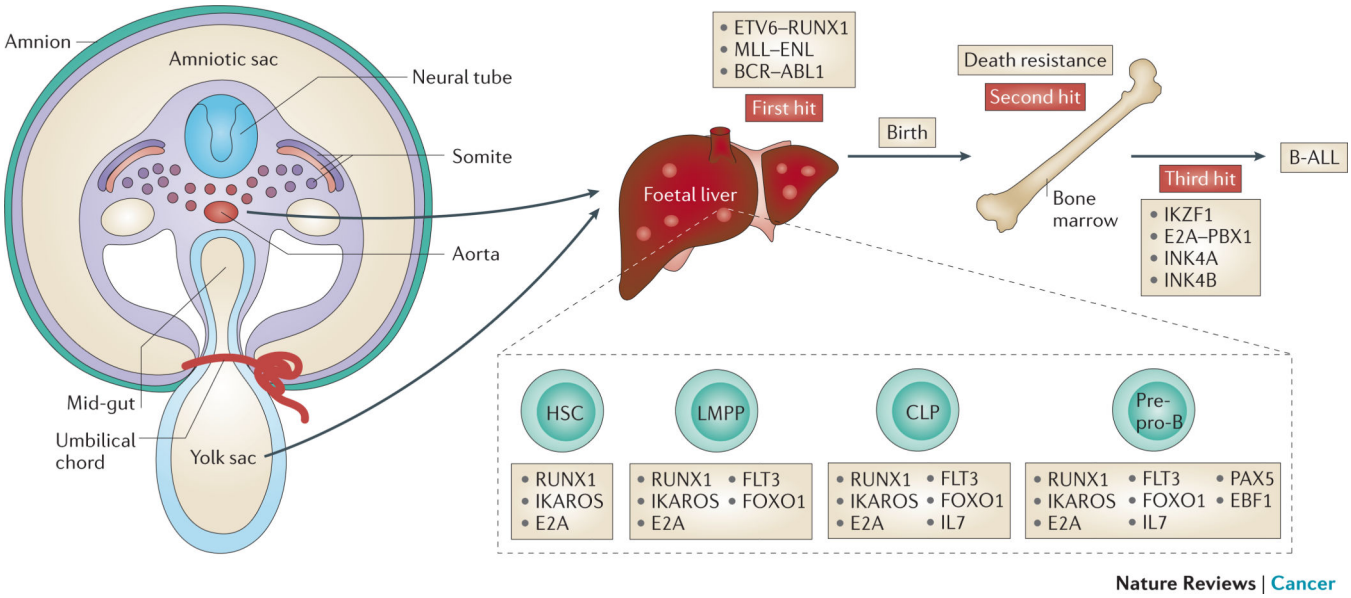

Figure 3. The development of B-ALL

The presence of specific prenatal oncogenic fusion proteins in haematopoietic stem cells (HSCs) or B progenitor cells combines with aberrant expression of transcription factors necessary for normal B cell development and maturation during the genesis of acute lymphoblastic leukaemia (B-ALL). Foetal haematopoiesis begins in the haemogenic endothelium formed from the yolk sac and aorto-gonadal-mesonephros (AGM), then localizes to the foetal liver, to finally reside in the bone marrow from the perinatal period. Several fusion genes (*ETV6-RUNX1, MLL-ENL, BCR-ABL*) present as clonal chromosome rearrangements at the point of diagnosis of B-ALL, represent the first hit as they have also been identified in blood samples at birth in children who later develop B-ALL. Since the incidence of the *ETV6-RUNX1* fusion gene is higher than the clinical incidence of B-ALL, some B-ALL rest cells must survive the perinatal period to undergo a third hit that leads to clinical presentation.

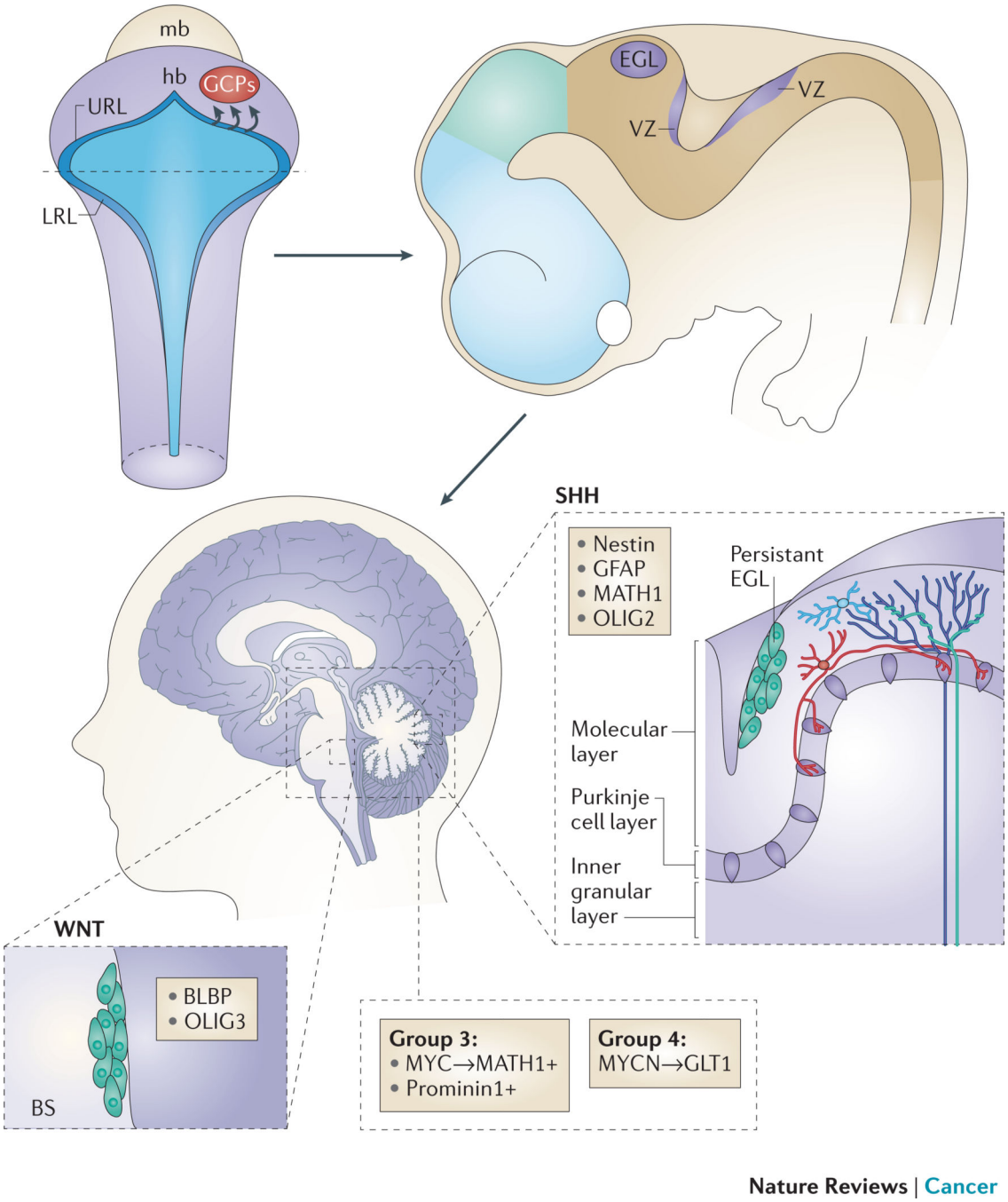

Figure 4. Cerebellar development and embryonal origin of medulloblastoma

The upper (anterior) rhombic lip (URL) is a germinal zone of proliferating granule cell precursors (GCPs) that migrate rostrally to form the external granular layer (EGL). A second germinal centre is the ventricular zone (VZ), which gives rise to Purkinje cells and several other types of cerebellar interneurons. Sonic hedgehog (SHH) tumours may arise from persistent cells of the EGL that have not migrated to the internal granular layer (IGL), while Wnt tumours may originate from persistent cells of the ventricular zone in the brainstem (BS). Group 3 and 4 medulloblastomas likely arise from neural stem cells of the hindbrain.

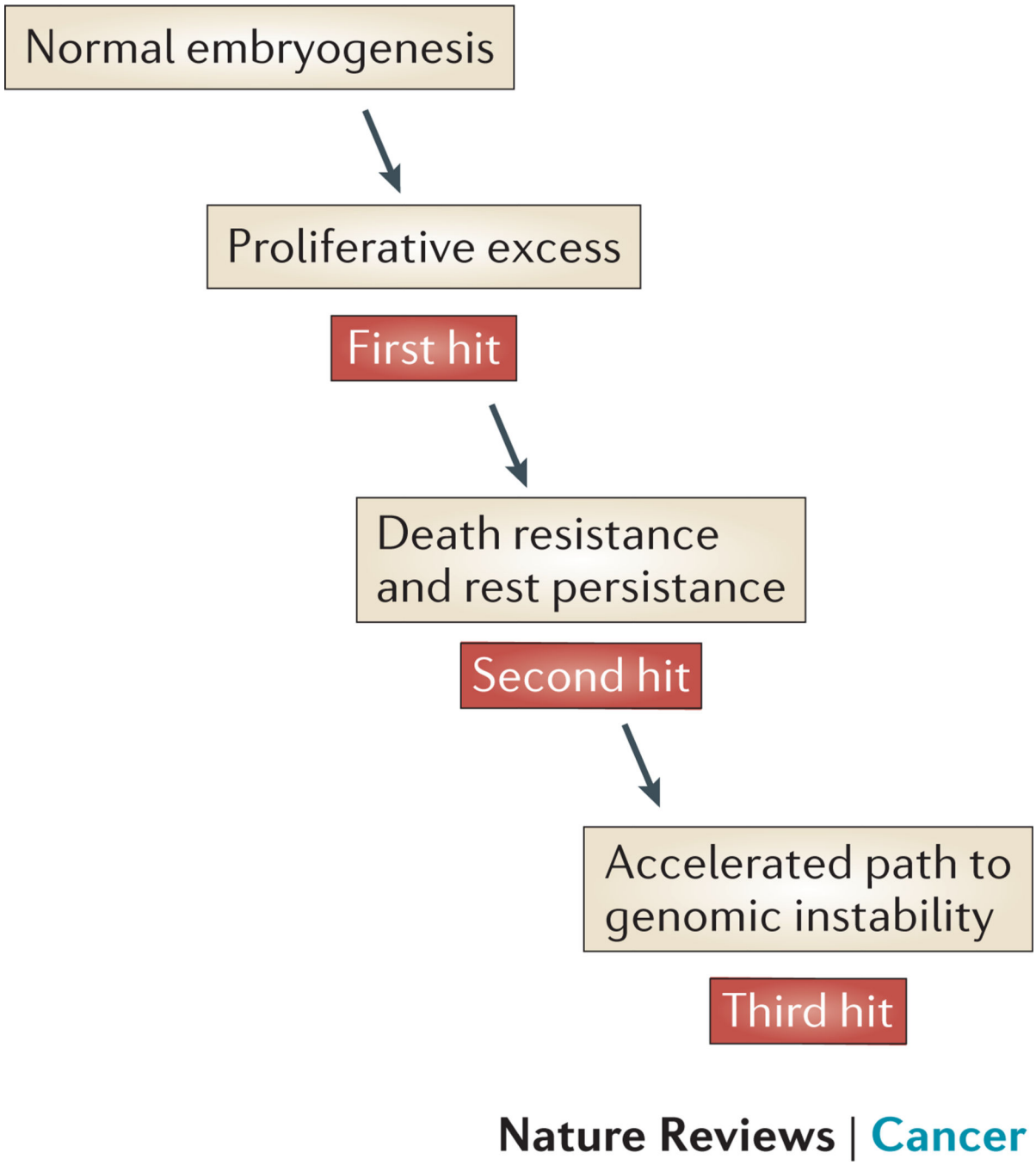

Figure 5. A model of embryonal tumorigenesis

We propose that each of the four embryonal malignancies with evidence of a prenatal origin (neuroblastoma, ML-DS, B-ALL, medulloblastoma) share common features: (i) a prenatal proliferative excess in the tissue of origin; (ii) a cell intrinsic mechanism for surviving the hostile early postnatal environment; (iii) an accelerated pathway toward genomic instability.

Table 1

Embryonal childhood malignancies

Malignancy	Tissue of Origin	Evidence for prenatal origin	
		Pre-Clinical	**Clinical**
Neuroblastoma	Neural crest sympathoadrenal progenitors (neuroblasts)	- Transgenic expression of genes involved in sympathoadrenal development (MYCN, ALK, LIN28B) recapitulate neuroblastoma[20, 33, 43]. - Animal modeling with *Th-MYCN* mice demonstrate rest formation prior to tumour formation due to resistance to developmental deletion signals[15, 19]. - Neural crest stem cell allografts generate neuroblastoma with oncogene (MYCN, ALK F1174L) transduction[44].	- Young age of incidence (<5 years)[12]. - Multifocal/bilateral primary tumours in tissues derived from the embryonic neural crest in some 20% patients[11]. - Normal foetal neuroblasts highly similar to tumour derived neuroblasts[45]. - Failure of complete sympathoadrenal maturation - Neuroblast rests and elevated catecholamines (neuroblastoma marker) in subclinical patients[46, 47, 172]. - Low stage neuroblastoma and rest cells frequently regress[4, 46].
Wilms Tumour	Primitive metanephrogenicblastema		- Young median age of incidence[173]. - Wilmstumour can be bilateral[173, 174]. - Inactivating mutations in kidney development gene WT1 predispose for Wilm'stumour[173]. - Nephrogenic rests adjacent to tumours[174]. - WT-1$^+$ nephrogenic rests found adjacent to WT-1$^+$ tumours[174]. - Nephrogenicrests can spontaneously regress[5, 175].
Medulloblastoma	Neural progenitors of the developing cerebellum/brainstem	- Aberrant SHH signaling through development recapitulates SHH subgroup medulloblastoma[114, 121, 122]. - SHH medulloblastoma modeling exhibit pre-malignant rests prior to tumour formation due to resistance to developmental cell deletion signals[117].	- SHH subtype associated with developmental disorder Gorlin Syndrome[111]. - Allelic loss on chromosome 9 (containing SHH pathway suppressor

Malignancy	Tissue of Origin	Evidence for prenatal origin	
		Pre-Clinical	**Clinical**
		- Activating mutations in β-catenin and aberrant WNT signaling in OLIG3+ neural precursors through brainstem development can recapitulate medulloblastoma[137]. - Animal modeling shows that cerebellar development genes (MYCN, MYC) can recapitulate medulloblastoma in SHH-independent medulloblastoma[126–128].	PTCH1) gene in 30% of tumours[113].
Retinoblastoma	Retinal progenitors of the developing eye	- Inactivation of retinal development genes Rb1 and p107 in retinal progenitors causes prolonged proliferation of progenitors through development and is sufficient to recapitulate retinoblastoma[176, 177].	- Familial cases are often bilateral, multifocal and usually occur in the first 2 years of postnatal life[143, 178]. - Retinal progenitor cell proliferation occurs only in foetal retina[178]. - Premature baby reported with retinoblastoma in early postnatal life[179]. - Patients are defined by familial and sporadic inactivation of retinal development gene Rb1[180].
Germ Cell Tumour (GCTs)	Primordial germ cells (PGGs) originate in yolk sac endoderm		- Subset of patients with early incidence < 4 years[181, 182]. - Teratomas can be found prenatally[183]. - Germline genetic predisposition have earlier onset[181]. - Bilateral GCTs[181]. - Genetic aberrations such as loss of 1p and gain of 1q are found in paediatric GCT which may reflect loss of tumour suppressor gene on 1p, involved in terminal differentiation of embryonic tissues[182]. - Malignant GCT component found within ‘benign’ teratomacomponent, reflects stages of malignant transformation[182].

Malignancy	Tissue of Origin	Evidence for prenatal origin	
		Pre-Clinical	**Clinical**
			- Carcinoma-in-situ cells resemble primordial germ cells in adult onset testicular GCT's[181].
B-ALL	Haematopoietic system (Lymphoid-primed multipotent progenitors LMPPs)	- Pre-leukaemic population detected *in utero* in *TEL-AML* transgenic mice[86, 87, 184].	- Twin studies of monozygotic twins with concordant leukaemia (TEL-AML1 positive pre-B ALL, infant ALL)[79, 81]. - Twin studies: clonal evolution of in utero pre-leukaemic population in neonatal blood spots in TEL-AML1[81, 86]. - Pre-leukaemic cells detected in neonatal blood spots for majority of pre-B ALL patients[2, 82].
TMD and ML-DS	Haematopoietic system (Megakaryocyte-Erythroid Progenitors MEPs)	- *GATA-1s* knock-in mouse model of TMD demonstrate transient hyperproliferation in megakaryocytic progenitors in embryonic yolk sac and foetal liver[67].	- Tissue of aborted 2nd trimester foetuses with Down syndrome demonstrating pathological increase in MEPs in foetal liver, prior to acquisition of somatic GATA1 mutation[54]. - GATA1 mutation detected *in utero*[66]. - *In utero* TMD diagnosis; condition present in preterm neonates[53]. - Median age of diagnosis 3–7 days postnatally, range 0–65 days[52, 53]. - TMD regresses prior to progression to ML-DS in a 25% of patients[49].
Malignant Rhabdoid tumours (MRTs)	Unknown cell of origin. Frequently arises in the kidney and central nervous system	- Heterozygous mutations of embryonic development regulator INI-1 in transgenic mice cause MRT predisposition and upon loss of heterozygosity manifests as MRTs[185].	- Fetal and neonatal MRTs frequently identified[183]. - Concomitant primary tumoursin both the kidney and brain[183]. - Familial or sporadic inactivation of INI-1 gene apparent in >95% of tumours[10, 186–188].