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# Is Genomic Imprinting Involved in the Pathogenesis of Pseudotriploid Neuroblastoma?

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## INTRODUCTION

Neuroblastoma is the most common solid tumor in children. It derives from the neural crest and originates from the sympathetic neuronal lineage [1-3]. At least two distinct biological-clinical entities can be distinguished [3-6]. One favorable subset occurs exclusively in infants and consists of early stages (I and II) as well as widespread disease (stage IV-S) at diagnosis. These tumors are commonly characterized by a hyperdiploid or pseudotriploid karyotype, but lack structural chromosome abnormalities. In particular, 1p abnormalities or N-*myc* gene amplification are not observed. Virtually all tumors identified with mass screening have belonged to these lower stages [4, 7, 8]. These patients show an excellent clinical outcome despite no or only minimal therapy. The other group of unfavorabled neuroblastomas is associated with older age and advanced stages (stages III and IV), and pseudodiploid karyotypes including 1p deletions and N-*myc* oncogene amplification [2, 9]. Their outcome remains poor despite aggressive multimodality therapy and bone marrow transplantation. It is interesting to note that favorable neuroblastomas rarely, if ever, evolve into unfavorable disease [3].

## Neuroblastoma in situ, neuroblastoma stage IV-S and spontaneous regression

The extraordinary biology and behavior of neuroblastoma spans from life-threatening progression to maturation into ganglioneuroblastoma and spontaneous regression of these neoplasms [10]. In fact, spontaneous regression is one of the most unusual aspects of neuroblastoma since it is between 10- to 100-fold more common than in any other human cancer [10]. The most dramatic regressions occur in infants with disseminated disease involving liver, skin and/or bone marrow, but not cortical bones or distal lymph nodes. To distinguish this clinical entity from the more usual progressive form of disseminated disease, stage IV, it has been termed stage IV-S [3]. The familial occurrence of

neuroblastoma in situ, ganglioneuroma, neuroblastoma IV-S and malignant neuroblastoma also underlines the close relationship of these tumors [11]. Neuroblastoma in situ is a lesion related to the modular clusters of neuroblasts. It is universially found in the adrenal glands of normal fetuses. The fact that it is detected incidentally at autopsy in neonates at a frequency far above the incidence of neuroblastoma indicates that most lesions will disappear before becoming clinically apparent [12]. These observations have therefore led to the notion that neuroblastoma in situ may not be a malignant tumor, but may rather represent a temporary anomaly in normal adrenal development or a hyperplastic nodule of mutant cells [10, 12].

The biological basis underlying spontaneous regression is still not understood. However, several hypotheses have dealt with this peculiar aspect [10, 12]. The proposed explanations include spontaneous maturation, programmed cell death and immunological antitumor effects. It has been proposed that stage IV-S patients may have a predisposing genetic defect since they also have an increased risk of developing progressive neuroblastoma later in life [11]. This defect may be either inherited and/or due to a somatic mutation of a primitive neuroblast. Knudson and Meadows [11] suggested that an inherited neuroblastoma gene can produce a spectrum of lesions by interfering with the normal development and normal progress of differentiation. Based on the notion that neoplastic transformation is a stepwise process, neuroblastoma in situ and neuroblastoma IV-S would thus represent tumors with only one-hit lesions, whereas malignant neuroblastoma may already have experienced two or more such hits. "Preneoplastic" neuroblasts in skin, liver and/or bone marrow derive from neural crest cells that normally migrate to those sites. Normally, these cells differentiate into Schwann cells or melanocytes or disappear. The "neuroblastoma mutation" temporarily arrests the development of these proliferating cells at an early stage. Delayed maturation ultimately transforms them into ganglioneuromas or neurofibromas or causes them to die. The latter may be due to programmed cell death, also known as apoptosis [10].

Apoptosis is most probably an active process in which the attenuation of genes and signals for cell survival take place. This concept suggests that all cells are constitutively prone to death by apoptosis, but are halted by trophic factors. In spontaneously regressing forms of neuroblastoma, the developmental timeswitch for apoptosis may therefore be delayed [10]. Only after activation of the apoptosis program do the cells die successfully and the tumors shrink. Varying rates of tumor regression are thus explained by the biological variation in the switching-off process. The occasional occurrence of a second hit in a single neuroblast might explain those rare instances in which apparent stage IV-S neuroblastomas first regress and then progress to true stage IV disease. Survival of one or a few damaged cells, promoted by trophic signals or by the expression of genes that inhibit apoptosis would be an essential first step in tumorigenesis [10].

The above explanations for the pathogenetic features of the spontaneously regressing neuroblastomas seem very plausible. However, they have not yet addressed the rather unique and peculiar genetic alterations characterizing these tissues. The benign clinical course of such tumors sharply contrast with generally accepted idea that tumors with chromosome abnomalities have already undergone malignant transformation. The mechanism leading to the increased number of chromosomes is currently unknown. However, once formed, both the hyperdiploid as well as the pseudotriploid karyotypes seem to be rather uniform and stable. Extending the above hypothese, I therefore suggest that

pseudotriploid neuroblastomas may originate from a tissue-confined residue of a diploid/triploid constitutional mosaic rather than from a specific, tumor-initiating mitotic error. This notion is based on the embryonic nature of the neoplasm, the early age of onset and, in particular, its ability to regress. These features strikingly resemble those encountered in transient myeloproliferative disease (TMD), a spontaneously regressing pseudoleukemia which predominantly occurs in individuals with a constitutional trisomy 21 [13]. The pathogenetic mechanism leading to this neoplasm may be, therefore, similar to the one extensively discussed in our hypothesis of a possible meiotic or early postzygotic origin of some trisomic neoplasms [13]. It may also be related to the occurrence of malformed mosaic tissues in patients with Beckwith-Wiedemann syndrome and uniparental disomy [14].

The general lack of gross structural chromosome abnormalities in hyperdiploid and pseudotripoloid neuroblastomas can be taken as an indication that specific DNA alterations are rather rare. Therefore, other causative factors have to be taken into consideration. That an epigenetic first step is responsible for the premalignant neoplasms in very young children is a particularly appealing idea, as conventional mutational mechanisms would probably not occur at high frequency or be reversible [15]. Disturbances in the growth and differentiation control mechanisms may thus be due to imprinting anomalies, exerted for example by the unequal distribution of paternally and maternally derived chromosomes or chromosomal regions and/or changes in the DNA methylation and expression patterns of particular genes. The maintenance and progression of neoplasms generally depend on ectopic and intrinsic factors. Deregulation of the paternally and maternally derived gene products could either enhance the proliferation capacity of the affected cells through a change in dosage or relative dosage of a set of genes or, in a similar process, block differentiation. During pregnancy, the responsiveness towards maternally derived growth promoters or differentiaton inhibitors may differ between diploid and triploid fetal tissues and, therefore, lead to the overgrowth of the abnormal cell line. The cessation of the maternal influence after birth together with the postnatal changes of gene expression, as for example that of the insulin-like growth factor II, may then trigger the regression of the abnormal tissue [16].

## Constitutional triploid and diploid/triploid mosaicism

Triploidy is estimated to occur in 1, 3% of recognized human conceptions [17, 18]. It consists of the presence of an extra haploid set of chromosomes for a total of 69 chromosomes. The two distinct types of embryonic/fetal phenotype and placental development depend on the parental origin of the additional set of chromosomes. A paternal extra haploid set correlates with relatively normal fetal growth and a large cystic placenta, whereas an additional maternal set is associated with intrauterine growth retardation, relative macrocephaly and a small noncystic placenta [17, 18]. In triploid conception, one pronucleus may be extruded at the first cleavage. Its degeneration will result in a diploid embryo, whereas its incorporation into subsequent cell divisions will give rise to a mosaic diploid/triploid conception [18].

Mosaic individuals with only a small triploid component will definitely have a higher chance for survival and will have far less pronounced phenotypic features than pure triploidies. Moreover, it is well known that in mosaicism the euploid cell line has a selec-

tive advantage and will therefore eventually outnumber the aneuploid one [13]. Nevertheless, such abnormal cells could survive in particular tissues in which their presence does not grossly interfere with normal development. To date, the phenotypes of at least 18 liveborn infants with diploid/triploid mosaicism have been reported [19]. Most showed a large number of common congenital abnormalities which, however, in comparison with full triploidy, were much milder. The clinical manifestations included mental and growth retardation, facial and body asymmetry, malformed and apparently low-set ears and syndactyly of fingers 3 and 4, whereas no gross malformations of the brain, heart or kidneys have been noted [19]. However, it should be pointed out that in a few cases, the main manifestations were restricted to slow growth and body asymmetry and that in the preponderant number of cases, the triploid cell line was only found in fibroblasts [19].

## Diagnostic strategies

It is surprising that virtually no information about the specific karyotype patterns of the hyperdiploid and pseudotripliod neuroblastomas is available. If my hypothesis is valid and in consideration of the influence of the extra haploid chromosome set on the biological behavior of incomplete moles and triploid conceptions [17, 18], it can be expected that the analysis of the chromosomal compositions of such triploid tissues will reveal nonrandom patterns with regard to the parental origin of the extra chromosomes. Whether the additional chromosomes are of uniparental or mixed biparental origin can be easily distinguished with molecular genetic means. Moreover, such studies would probably help to clarify the as yet unclear mechanisms which generate such abnormal karyotypes. Thus, they could reveal whether hyperdiploid and pseudotriploid karyotypes are the product of simultaneous or sequential events (or an event) as well as whether they result from meiotic, early postzygotic or mitotic nondisjunction errors. The study of clonal evolution in different abnormal tissues of individual cases might disclose whether tumor spreading is a true metastatic process or rather occurs as physiological migration of neural crest cells during normal fetal development.

Careful analyses of particular clinical aspects could also be rewarding. It is intriguing that in chidren with malignancies, an increased prevalence of minor anomalies has been recognized for a long time [6, 20]. Developmental abnormalities of the placenta and the fetus as well as the presence of subtle phenotypic anomalies, such as delayed growth, a triangular and/or asymmetric face, micrognathia, finger and/or toe syndactyly, clinodactyly, single transverse palmar creases, genital anomalies and hypotonia could point to a possible underlying constitutional mosaicism. In addition, unusual skin pigmentation patterns, such as patchy cutaneous hyperpigmentation and or hypopigmentation, have been found to be helpful clues in detecting low-grade mosaicism in chromosomal disorders.

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