

Übersicht

Significance of Polyploidy in Megakaryocytes and Other Cells in Health and Tumor Disease *

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Summary. Polyploidy – the doubling of chromosome sets of cells caused by a stop of mitosis at different levels of the mitotic cycle – is a phenomenon widely observed in plants, protozoa, metazoa, and animals. In man obligate polyploid tissues are found in liver parenchyma, heart muscle cells, and bone marrow megakaryocytes. Polyploidy occurs mostly in stable and highly differentiated cells and tissues. Besides age, stimulation of proliferation and increased metabolic function lead to polyploidization in these organs. Aneuploidy, however, is exclusively found in tumor cells.

Megakaryocyte differentiation and polyploidy are controlled by thrombopoietin-like activities, of which the loci of production are still unknown. Megakaryocytes are unique among polyploid mammal cells. On the precursor level they maintain their proliferative activity independently of the mammal's age. Once having entered the incomplete mitotic cycle they stop cytokinesis and develop into highly polyploid cells. Polyploidization of megakaryocytes is the basic requirement for establishing highly effective hemostasis in mammals, which exhibit blood circulation based on high blood pressures.

Every polyploidization results in increased production of membrane materials with which the platelet becomes endowed. By shedding cytoplasmic fragments approximately 3000 platelets are set free from a 32c megakaryocyte, compared with only 16 nucleated thrombocytes by mitotic divi-

sion. There is further evidence that the heterogeneity of platelets mostly depends on the different polyploidy classes of the megakaryocytes from which they are derived. Changes in the polyploidy pattern of megakaryocytes could therefore have consequences for hemostatic disorders in several human diseases, particularly in malignancy.

Key words: Polyploidy – Megakaryocytes – Megakaryocytopoiesis – Malignancy

Definition and Historical Review

Polyploidization is defined as the conversion of cells or organisms from the haploid or diploid state to a polyploid state [153]. In this state they may have, for instance, twice (tetraploid), four times (octaploid), or other multiples of the diploid DNA content. Long before DNA could be measured by DNA cytophotometry, polyploid mitoses were observed which revealed increased numbers of chromosome sets, instead of two as in diploids. The term polyploidy was first introduced by the botanists Strassburger [175] and Winkler [196]. The latter succeeded in growing tetraploid plants by clipping and grafting the apical shoots of *Solanaceae*, and thus provoking a generation of adventitious shoots. Winkler realized that reimplanted tetraploid shoots grew to larger forms (“gigas species”) with thicker stems, broader leaves, and larger inflorescences. He correctly attributed this characteristic shape to the doubling of the number of chromosomes in these species.

General Aspects and Prevalence

Since Strassburger and Winkler polyploidy has largely been used in plant breeding. Particularly

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Abbreviations: n=haploid chromosome set (number of chromosomes actually counted); c=haploid DNA content (measured by cytophotometry); M=mitosis; C-Mitosis=colchicine mitosis; G₀, G₁, G₂, S=phases of the mitotic cycle; Meg CSF=megakaryocyte colony stimulating factor(s); TSF=thrombocytopoiesis stimulating factor(s); CFU-Meg=megakaryocyte colony forming unit

in growing ornamental plants, breeding of polyploid species has brought striking success [81]. The hope of breeding polyploid useful plants, however, has not been completely realized because many useful plants are already polyploid variants of diploid wild species. When further polyploidization is induced some drawbacks are accentuated. Above all decreased reproduction potential is one of the main disadvantages. Thus, with increasing polyploidy the number of fruits is often diminished, although the single fruits are generally larger than those of diploids. Somatic polyploidy in plants often stops at the $4c$ and always before the $16c$ level. Most of the higher polyploid variants are again smaller and not viable. Although only about 10% of all plant species [55] have been examined for their somatic polyploidy, estimates based on this number reveal a percentage for polyploid spermatophyta between 30% and 35%. For wild species there is a distinct south-north gradient. The further north the location, the higher the number of polyploid species becomes [55].

In addition to plants, polyploid nuclei have been found on a large scale in protozoa, metazoa, and animals. The list is apparently only limited by the range of application of DNA cytophotometry.

Mechanisms

The mechanism which forms polyploid cells is partially understood. It is displayed in Fig. 1, which shows the cell cycle with various mechanisms of genome multiplication. However, the regulatory systems, causes, and benefit of polyploidization have been until now a matter of intense discussion.

Regarding the mitotic cycle, mitosis can stop at different phases, thus leading to polyploid cells.

Endomitosis – Endoreduplication

Endomitosis is characterized by a blockage of the prophase, without dissolving the nuclear membrane. According to the original definition of Geitler [49], the term endomitosis is reserved exclusively for this form of DNA replication. Endomitosis is common in differentiated tissues of insects and higher plants [3, 179].

In human tissues endomitosis has been observed in trophoblast cells of human placenta [160] and in human cancer cells [177].

If the cell does not enter the mitotic cycle at all and remains in the G_2 phase, an “endoreduplication” of DNA occurs. This was first observed in ascites tumors of the mouse and in eukaryotes;

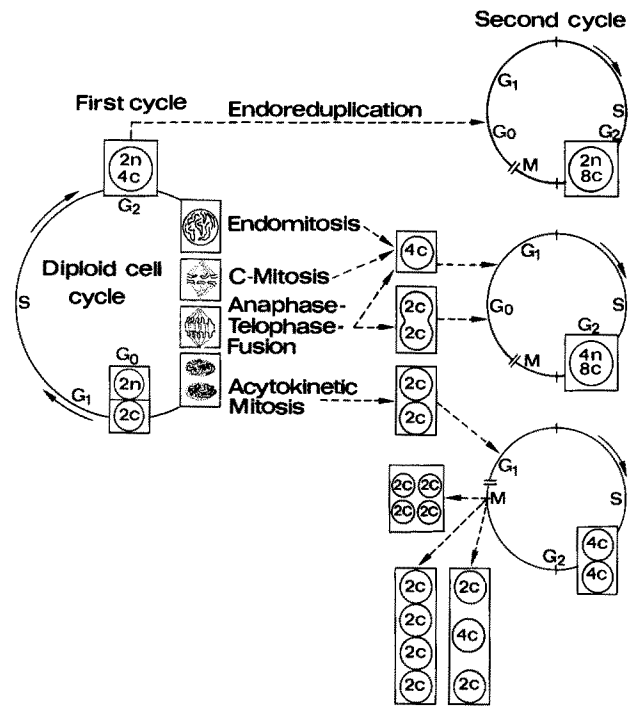


Fig. 1. At the beginning of the normal diploid cell cycle DNA synthesis ends when the $4c$ level is reached. During this premitotic phase the number of chromosomes remains diploid ($2n$), but the DNA content has already doubled ($4c$). If the cell does not now enter the mitotic cycle, endoreduplication can begin. As the doubled chromatids remain together lengthwise, the number of chromosomes always remains “diploid” ($2n$), but the DNA content doubles with every endoreduplication step ($4c$, $8c$, ...). In the case of endomitosis the nuclear membrane is not dissolved, but the chromatids separate and consequently the chromosome number is doubled. In the case of a blockage during the metaphase (C-mitosis) or early anaphase, $4c$ cells develop which can again double their DNA content in a second cycle. In the case of anaphase or telophase fusion, the dumbbell-shaped nuclei can look like two nuclei melting together or separating. Acytokinesis finally leads to binucleate cells which can again double their DNA content by entering a second cycle with several possibilities of polynucleate cells (cells with four nuclei or more, e.g., in liver parenchyma or in urothelial cells). In the heart muscle cells, telophases of two neighboring mitotic nuclei can again melt together, leading, for instance, to a $2c$ - $4c$ - $2c$ -cell

it can also be induced by chemical agents [13, 65, 89, 109, 166, 167]. A special form of such a G_2 block leads to giant chromosomes by repeated duplication but nondisjunction of the chromosomes. The chromatids remain together lengthwise. Such cases of “polyteny” are well known in salivary glands of *Diptera* [9] and in giant neurons of certain mollusks with DNA amounts of more than 300000 haploid units [29, 85].

Endoreduplication and endomitosis differ from each other. In endomitosis mitosis-like changes can be observed. In an endoreduplication cycle, however, no mitosis-like structural changes can be seen

in the nucleus. Some reports of endoreduplication have been called into question, and the usefulness of differentiation between endoreduplication and endomitosis has been in doubt [113]. However, other studies indicate that real cases of endoreduplication do occur in plant cells [96] and animal cells [127, 155].

Blockage During the Metaphase

Mitosis can also stop in the metaphase, i.e., the middle of the mitotic cycle. The best example of this is the experimental blockage of mitosis by colchicine, which impedes the spindle apparatus but not the separation of chromosomes [88]. Examples of DNA reduplication representing this kind of formation of highly polyploid nuclei are found in facultative and obligate polyploid human tissues, as described below.

Binucleate Cells

If mitosis is interrupted during the telophase, binucleate cells develop as a result of a lack of cytokinesis (acytokinetic mitosis). Binucleate cells are very common in nearly every tissue of mammals. They are especially typical in urothelial cells [182], salivary glands, and vegetative ganglia. Binucleate cells amount to 50%–70% of all hepatocytes in liver parenchyma [19].

Polyploidy of Human Cells and Tissues

Polyploidy in human tissues is well known in liver parenchyma and heart muscle cells, where polyploidy occurs regularly beyond a certain age, i.e., the developmental stage. Therefore, these organs are *obligate polyploid* tissues. Polyploidy has also been reported in thyrocytes, epithelial cells of the seminal vesicles, smooth muscle cells, tubular cells, adrenal cells, and the human trophoblast. In all these organs polyploidy is only inconstantly observed under certain circumstances such as advanced age or various functional and hormonal stimuli. These are *facultative polyploid* organs. To elucidate the causes of polyploidy we would like to discuss in more detail both types of polyploid organs.

Facultative Polyploid Cells and Tissues

Seminal Vesicles

It was demonstrated by cytophotometric investigation that epithelial cells of human seminal vesicles were usually diploid in healthy boys. A great

number of tetraploid cells appeared by the end of the second decade, and octoploid and hexadecaploid nuclei appeared in the smears of elderly men [110, 111]. The correlation of polyploid nuclei in human seminal vesicle cells with increasing age was confirmed by Wittstock and Kirchner [198], Paulini and Sonntag [128], and Heide [62]. Wittstock and Kirchner [198] did not see any correlation of higher ploidy in the aged with prostatic hypertrophy and spermatocystitis. They concluded, like Paulini and Sonntag [128], that polyploidy might be a sign of regressive changes of cells and tissues in the elderly. Mohr et al. [110], however, assumed that polyploid cells in the adult and aging organism have a higher functional activity than diploid cells, as polyploidization is preferentially observed in stable, nonproliferative cells and tissues. These contrasting points of view are typical in interpreting polyploidy of any human tissues.

Thyroid

In the thyroid more than 96% of the thyrocytes are diploid throughout life. There is only a slight increase of 8c and 16c nuclei with age [185]. On the other hand, in hyperthyroidism and in goiters a shift to higher ploidy values could be observed [14, 28, 57, 106, 144, 184]. In goiters with regressive changes, however, no alteration of the usual ploidy pattern was detected [54]. At first glance it seems, therefore, that polyploidization in the thyroid is due to hormonal hyperfunction. Bjelkenkrantz et al. [14], however, found in combined autoradiographic and cytophotometric studies no polyploid nuclei in thyroid lesions with clear-cut biochemical hyperfunction, although they measured polyploid thyrocytes in other regions of toxic goiters. Polyploidy is likewise not influenced by thyreostatic drugs [184].

Other Organs

In adrenal cells a significant increase of 4c nuclei is observed in patients above 50 years of age [54]; in rats only older animals show an increase in ploidy of tubular cells after unilateral nephrectomy [129]. In smooth muscle cells of the uterus, however, polyploid nuclei arise only during pregnancy [63], which indicates that polyploidy is a result of increased functional (hormonal) activity.

Obligate Polyploid Cells and Tissues

Liver

The parenchyma in the liver contains only diploid nuclei from birth to the age of about 12 years.

A small tetraploid collective, appearing in some cases before 14 years of age, is always demonstrable at 40 years of age, and increases from that time to senility. Large nuclei of octaploid and higher values first appearing at about 20 years of age show an increase in number in man at about 60 years, and reach their maximum after 70 years of age [2, 8, 33, 176]. Brodsky and Uryvaeva [19] showed that mean ploidy of hepatocytes increases, and the amount of 2c hepatocytes decreases, with increasing life spans in mice. Intensive proliferation of diploid hepatocytes occurs only in baby mice. After about 1 month the diploid cells cease to proliferate and transform into binucleate and other polyploid cells. The weight of the rat liver increases almost 30 times within the first 2 years of life, but the number of liver cells increases disproportionately to the weight. The postnatal growth of murine liver parenchyma is, therefore, mainly due to polyploidization and cell enlargement. Several authors have demonstrated that liver cells answer to stress by drugs such as phenobarbital [17, 115] and isoprenaline [51], or to partial hepatectomy [19] and bacterial stimuli [164], by an increase in polyploidization of hepatocytes. A number of polyploid nuclei greater than expected are also present in hepatitis, posthepatic conditions, and cirrhotoses [2].

It has further been shown that proliferative potentials of hepatocytes seem to be influenced by age, for example, restoration of liver parenchyma is slower in aged than in young mice [20]. Generally the growth fraction and proliferation capacity of liver parenchyma decreases with age, while polyploidy increases [165, 168, 173, 174]. Thus, proliferation of liver parenchyma cells is mainly confined to the postnatal period in mice and to the first decade in man. With the decrease of proliferation of 2c cells an increase in polyploid cells occurs, reaching higher ploidy values in the aged. Stimulation of proliferation and increased metabolic function also lead to polyploidization of hepatocytes. The degree of this polyploidization, although roughly correlated with age, does not seem to be due exclusively to age-related defects of the mitotic cycle. It seems also to be a response to several stimuli, and to be influenced by the decreasing ability of cell division with increasing age.

Heart Muscle Cells

Polyploidization of the heart muscle cells was first observed by Sandritter and Scomazzoni [159]. It happens in children at about the age of 7 with a heart weight of between 100 and 150 g. Beyond

this age about two-thirds of the nuclei of the left ventricular wall are polyploid, with DNA contents of 4c, 8c, and 16c [140], and do not alter polyploidization patterns under normal conditions for the rest of life. In the right ventricle 2c remains the main ploidy class. Mitoses with cytokinesis are mainly confined to the embryonal lifetime. After birth mitoses occur with a rapidly declining frequency during the first month in dwarf pigs [84] and can be observed in perinatal human hearts [162]. These observations lead to rejection of the theory of amitosis in heart muscle cells [143]. Myocardial polyploidization, however, is only characteristic for some higher mammals such as swine, monkeys, and primates [56, 142, 159]. In DNA measurements of heart muscle cells of mice [139], rats [108, 146], turkeys [141], and cows [1], no polyploidization could be observed. In human hearts under pathological conditions, however, e.g., hypertrophy due to hypertension or heart valve diseases, congenital malformation of the great vessels [140, 146], and after myocardial infarction [37], an increase in polyploidy is observed even in early childhood. In cases of pulmonary hypertension distinct polyploidization also occurs in the myocardium of the right ventricle [156]. However, there are individual differences in response; the shift to higher ploidy values of heart muscle cells depends mainly on the intensity and the duration of the stimuli leading to hypertrophy [37, 140, 145, 156]. Polyploidization, however, could not be seen in rats after experimentally induced renal hypertension [82].

In summary, polyploidization in heart muscle cells in primates and pigs seems to be a phenomenon which increases with age and depends on loss of proliferative activity shortly after birth. This, however, can hardly be explained by degenerative mitotic cells mechanisms because the main polyploidization takes place in children aged about 7 years, and remains on a stable level until death unless diseases leading to myocardial hypertrophy occur. Polyploidization in the heart also seems to be a matter of evolution.

In contrast to liver cells, hearts of lower mammals and birds actually do not develop polyploidy of heart muscle cells, even under induced hypertrophy. Liver tissue is also never polyploidized in cats [180].

Tumors

A summary of polyploidization in human tissues would be incomplete without mentioning quantitative DNA alterations in human cancer cells. Al-

though the term ploidy is often unfortunately used in this connection, aneuploidy, e.g., hypodiploidy or DNA values of 2.5, 5c, etc., is actually meant. In addition to these aneuploid stem-lines, veritable polyploid nuclei certainly occur to some extent in human tumors. Aneuploidy is observed in varying percentages of human tumors [7, 17]; thus, lack of aneuploidy is not proof of benignity [6]. Leukemias and malignant thyroid tumors are often strongly diploid [54, 57, 66]. Conversely, however, the proof of aneuploidy is a strong indication of malignancy or a precancerous condition, since nonmalignant tissue alterations are not associated with DNA aneuploidy [7].

Polyploidy of Megakaryocytes and Regulation of Megakaryocytopoiesis

Mitotic Cycle

Polyploidy of megakaryocytes had been recognized by Bizzozero [15] and Arnold [5]. Heidenhain [64] gave one of the most accurate descriptions of megakaryocyte morphology. Observing megakaryocyte mitoses, he recognized that he could never see mitotic figures passing beyond metaphase. The degree of polyploidy could be estimated by the amount of nuclear lobes [71] or determined by counting the chromosomes in mitoses [183]. Precise measurements of DNA content of megakaryocytes were first carried out on a large scale by application of Feulgen cytophotometry [45, 86]. Mitoses of megakaryocytes can be specifically inhibited by the application of the metaphase blocker colchicine [154]. This suggests that the spindle apparatus plays a certain role in polyploidization of megakaryocytes. Additional electron microscope findings confirm that megakaryocytes pass entirely through the prophase [12] and enter the metaphase (Fig. 2). Therefore, mitoses of megakaryocytes are thought to stop somewhere during the late metaphase or early anaphase. Mitotic figures of megakaryocytes are rarely seen in bone marrow smears, but this is undoubtedly due to the relative rareness of megakaryocytes, which comprise only about 0.05% [93] in the bone marrow compared with other blood cells. Japa [71], Rothlin and Undritz [157], and Weicker and Nöller [183] studied mitotic figures of megakaryocytes in the bone marrow smears of adults and children. The latter examining more than 10000 megakaryocytes observed 50–55 mitotic figures and calculated a rate of 0.5%–1.0% mitoses in megakaryocytes. This fits precisely with the data of Odell and coworkers [123], who determined the percentage of labeled mitotic figures of

murine megakaryocytes at intervals after a single injection of tritiated thymidine. They calculated an average value of megakaryocytes in the mitotic cycle of 0.7% and an average time for mitoses of about 45 min. Time for the S phase was 7.5 h, and 30 min for the G₂ phase. As the time for the total cell cycle was determined as 9.5 h, which is comparable with generation times for other mammalian hemopoietic cells [18, 72, 158], nearly no time is left for the G₁ phase.

A more accurate understanding of cell kinetics of the megakaryocyte cell line was obtained in the autoradiographic studies in rats made by Feinendegen et al. [42]. They showed that the first megakaryocytes labeled, about 35%, were among the immature megakaryoblasts (type I) 1 h after the injection of H³-thymidine. Labeling of only 10% of promegakaryocytes and no labeling of mature megakaryocytes was observed. Comparable labeling of promegakaryoblasts (type II) and mature megakaryocytes, approximately 35%, followed 8 and 18 h after the injection. These findings were confirmed later by Ebbe and Stohlman [34], Cooney and Smith [30], Odell and Jackson [18], and Queisser et al. [147], who showed that DNA synthesis by polyploidization is largely confined to the immature stages of recognizable megakaryocytes, i.e., the megakaryoblasts.

Acute Thrombocytopenia

More about the regulation of megakaryocytopoiesis was learned from the studies of megakaryocytes in animals with induced thrombocytopenia. It was already known from earlier studies that changes of the number of megakaryocytes in bone marrow occur following the induction of thrombocytopenia by hemolytic agents [21] and antiplatelet serum [11, 171, 197]. These findings were confirmed by several authors [32, 99, 121]. Harker [58, 59] and Ebbe [35, 36] reported both an increase in number and size of megakaryocytes in thrombocytopenic rats. On the other hand, thrombocytosis induced by transfusion of viable platelets was reported to result in a decrease of both number and size of megakaryocytes [59, 122].

DNA Content of Megakaryocytes in Acute Thrombocytopenia

It was Penington and Olsen [134] who first determined polyploidy of megakaryocytes by applying DNA cytophotometry. They observed that stimulation of platelet production in rats resulted in an increased polyploidy of megakaryocytes 2 days

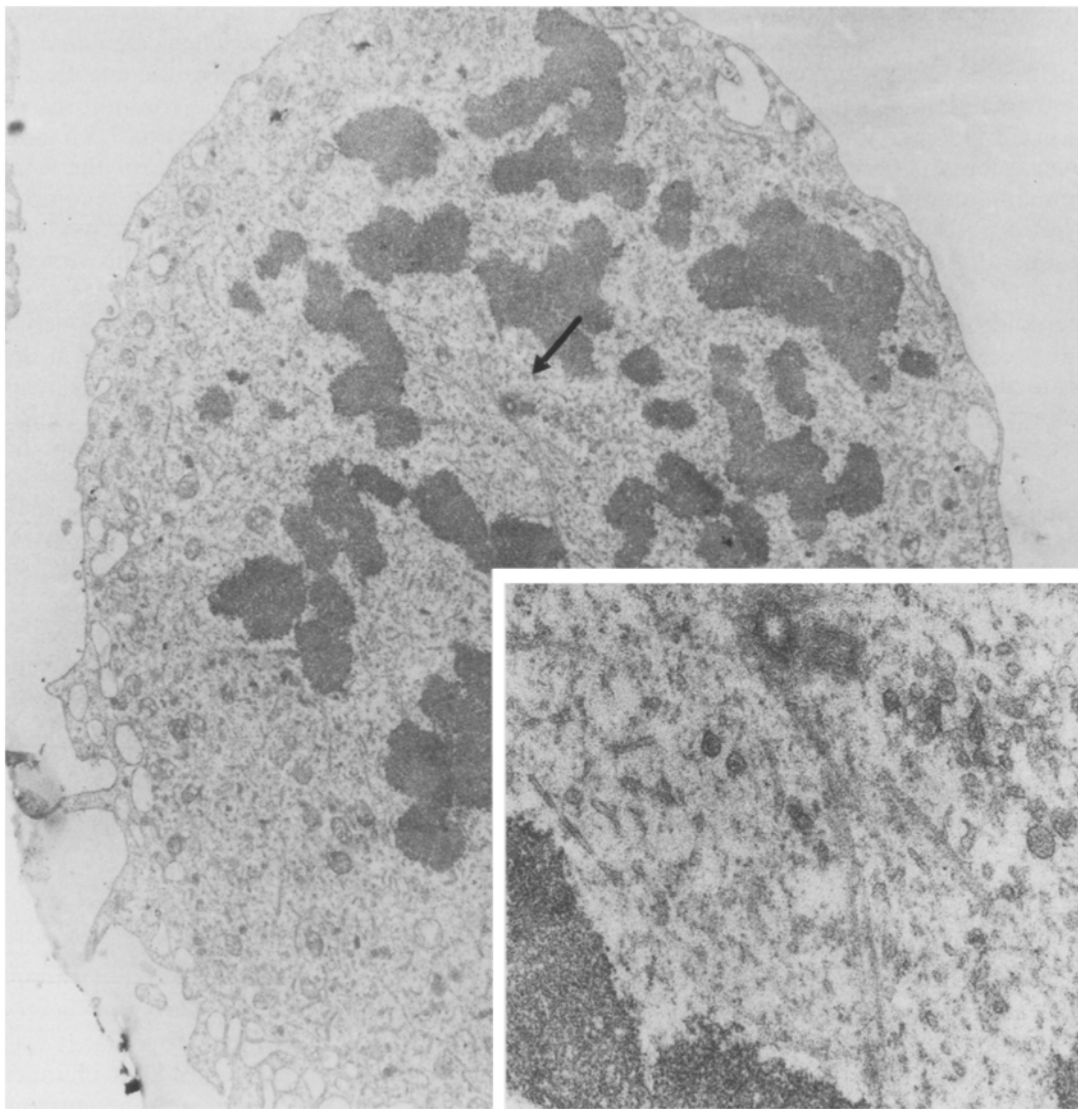


Fig. 2. Electron microscopic appearance of mitosis (metaphase) of a megakaryoblast with clear vacuoles. Only very few cytoplasmic granules are present. Arrow points to the centriole with parts of the spindle apparatus ($\times 7200$). See enlarged section ($\times 31200$)

after the application of antiplatelet serum. This effect could not be detected after sustained thrombocytopenia. Counting the number of megakaryocytes in acute thrombocytopenia, they observed an increase in megakaryocytes which was even more pronounced in rats with prolonged thrombocytopenia. Conversely, platelet transfusion resulted in lower polyploidy levels and a decrease in number of megakaryocytes in bone marrow and spleen. The count of megakaryocyte lobes, however, proved to be an inadequate method for determining DNA content. The observations of Penington and Olsen [134] were later confirmed by Odell et al. [125] and Trowbridge and Martin [178] in similar experimental studies. Indirect confirmation was obtained in studies made by Burstein [25], which

showed that megakaryocyte volume – which implies an increase in ploidy – increases in 24–65 h after induced thrombocytopenia. The studies by Odell and coworkers [119, 120, 124] produced additional results that confirmed the data of Penington and Olsen [134]. They could demonstrate that induction of thrombocytopenia resulted in an increase in the mitotic index of megakaryocytes, and that the mitotic index and elevation of platelet count were more pronounced and rapid after severe rather than moderate thrombocytopenia. They further showed that, after induction of thrombocytopenia, an increase of immature highly polyploid megakaryocytes occurs which is followed by an increase of mature forms with higher polyploidy. In another study, Odell et al. [126]

demonstrated an increased endomitotic index of megakaryocytes in rats treated with plasma of thrombocytopenic animals, whereas plasma infusion from rats with normal platelet counts had no effect. In numerous experiments it was further shown that plasma from thrombocytopenic rats [31, 60], rabbits [39, 169], and mice [31, 114] induced platelet production in the transfused recipient of the same species.

Thrombocytopoiesis Stimulating Factor (TSF)

From the above results it can be concluded that one or more factors acting on megakaryocyte polyploidy and cytoplasmic differentiation are present in or induced by the plasma of thrombocytopenic animals. This factor or factors undoubtedly induces an increase in number, polyploidy, and size of megakaryocytes, thus leading to an increase in platelet production. This factor is called thrombopoietin or thrombocytopoiesis stimulating factor (activity; TSF) because multiple stimulators of thrombocytopoiesis have been detected from different sources. Thrombocytopoiesis-stimulating activity was evaluated with either 75-selenomethionine and $\text{Na}_2^{35}\text{SO}_4$, which were incorporated into the cytoplasm of megakaryocytes. A relationship between the degree of megakaryocytopoiesis and the rate of incorporated isotopes into the cytoplasm of megakaryocytes could then be demonstrated [41, 59, 130, 169]. Several studies proved that certain fractions obtained from the plasma of thrombocytopenic animals have thrombocytopoiesis-stimulating activity [39, 90, 102, 104]. This activity has also been demonstrated in supernatants of a human embryonic kidney cell line [103], in a medium conditioned by cells of a murine myelomonocytic leukemic cell line (WEHI-3) [107], and in the serum-free supernatant of an adherent cell population of peritoneal exudate obtained from C57BL/6 mice [189], as well as in the urine of patients with idiopathic thrombocytopenic purpura [76]. Thrombopoietin, a glycoprotein similar to erythropoietin, was purified approximately 1000-fold by Evatt and coworkers [40] from the plasma of thrombocytopenic rabbits. There is strong evidence that thrombopoietin and erythropoietin are two different hormones, although thrombocytopoietic effects of erythropoietin have been reported (for more details, see Levin and Evatt [90], Geissler et al. [46], and Evatt et al. [41]). The locus of main production of thrombopoietin is still unknown, although results of partial hepatectomy [61, 170] in rats, and the phenomenon of thrombocytopenia in chronic liver diseases,

especially in liver cirrhosis [80, 161, 191], suggest that the liver might be one of the sources of thrombopoietin-like activity.

Culture Systems and Megakaryocyte Colony Stimulating Factor (Meg CSF)

With the development of in vitro culture systems for megakaryocytes, more light was shed upon regulation of megakaryocytopoiesis. All in vitro methods developed to date (plasma clot technique [105]; soft agar system [107]; microagar culture system [47] require the presence of fetal calf, horse, or human AB serum. An additional stimulus supplied by various "conditioned media" [23, 47, 107, 133, 186] is also needed. In these in vitro assays regulatory mechanisms of megakaryocytopoiesis to various stimuli could be studied. Williams et al. [188] developed an in vitro assay with two distinct factors necessary for megakaryocyte colony development. Concentrations of thrombocytopoiesis stimulating factor (TSF) did not directly stimulate the growth of colony forming units of megakaryocytes (CFU-Meg), but increased the frequency of CFU-Meg when added to the cultures with a constant amount of megakaryocyte colony stimulating factor (Meg CSF) [186]. Williams concluded that this second factor was needed for megakaryocyte proliferation acting on the proliferation stage of megakaryocyte precursor cells. This megakaryocyte colony stimulating factor (Meg CSF) was found by Hoffman et al. [67] in sera of 11 patients with hypomegakaryocytic thrombocytopenia, and was tested in a plasma clot culture system. It enhanced the formation of CFU-Meg derived colonies by as much as 1840%. Moreover, Hoffman et al. [68] succeeded in purifying Meg CSF from these patients, which resulted in an increase in specific activity by 3489-fold. Purified Meg CSF was capable of promoting megakaryocyte colony formation at a concentration of 7.6×10^{-8} M. Meg CSF was also found and characterized by Kawakita et al. in the urine of patients with idiopathic thrombocytopenic purpura [77] and other thrombocytopoietic disorders [78].

Polyploidy of Megakaryocytes in Culture Systems

In other experimental studies numerous investigators could show that thrombocytopoiesis stimulating factor (TSF), or megakaryocyte potentiator activity [189], obtained from various sources did not affect the number of megakaryocytes per colony, but did increase the DNA content of developing megakaryocyte colonies [92, 97, 189]. Levin et al. [91] measured DNA content from megakaryocyte

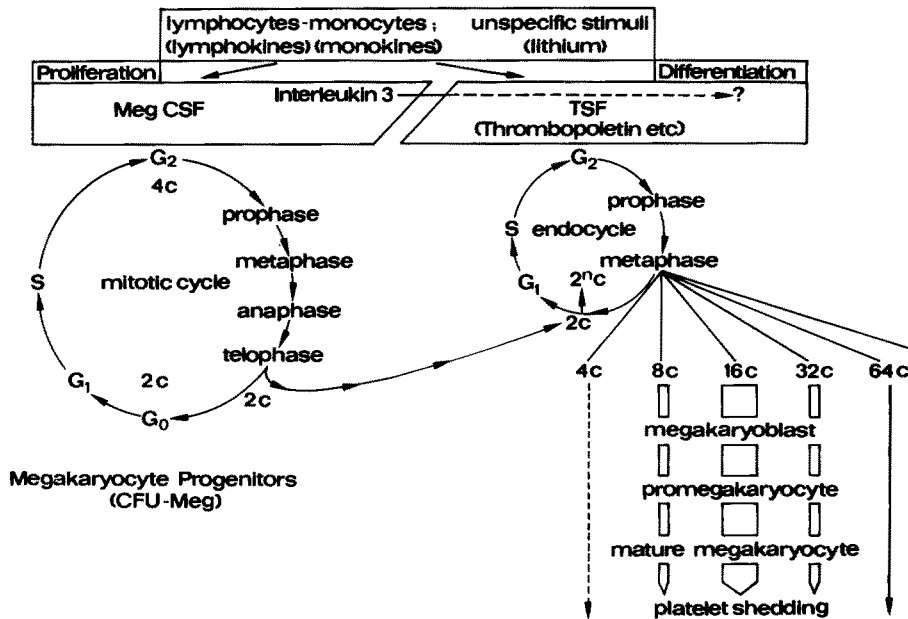


Fig. 3. Regulation of megakaryocytopoiesis (see text)

colonies in cultures of bone marrow taken from normal and thrombocytopenic mice. In the control animals they observed two types of megakaryocyte colonies; one had a mean ploidy level of 16.8c per cell (which is exactly the mean ploidy level in normal mammal bone marrow), and the other colony had a considerably lower mean ploidy level (6.8c/cell). In cultured bone marrow taken from mice 24–48 h after induction of thrombocytopenia, the megakaryocyte colonies with a mean ploidy of 16.8c/cell showed a significant shift to higher ploidy values (mean, 21.5c/cell), with 6% 64c megakaryocytes. In contrast, Chatelain and Burstein [26], using the same medium, could not observe ploidy changes of cultured megakaryocytes after short-term exposure to a thrombocytopenic environment. Under the same culture conditions, Levin et al. [92] studied the effect of thrombopoietin derived from plasma of thrombocytopenic rabbits on cultured murine bone marrow cells. He detected a slight but not significant increase in ploidy values of the large cell colonies. Long and coworkers [97], although not measuring DNA content, observed the development of round nucleated immature megakaryocytes into large mature megakaryocytes when cultured with thrombopoietin stimulating factor derived from a human embryonic kidney cell line.

Role of Erythropoietin

Research on the role of erythropoietin as a megakaryocyte colony stimulating factor (Meg CSF) is

partially contradictory. Whereas McLeod et al. [105], Vainschenker [181], and Freedman et al. [43] observed that high concentrations of erythropoietin increased the frequency of megakaryocyte colony forming unit (CFU-Meg) cells in plasma-clot cultures, Mazur et al. [101], Levin et al. [92], and Williams et al. [190] found very little or no effect of various erythropoietin preparations in enhancing CFU-Meg. The contradictory results concerning the effect of erythropoietin on megakaryocytopoiesis may be due to the use of contaminated hormone. However, thrombopoietin and erythropoietin probably have similar chemical structures; high doses of erythropoietin could bind to the receptor sites of thrombopoietin, thus mimicking its effects [10].

Taken together, these studies have finally led to the *two-factor hypothesis* for regulation of megakaryocytopoiesis, which is similar to that of granulocyto- or erythrocytopoiesis. This hypothesis states that two factors are required for regulation of megakaryocytopoiesis (Fig. 3). One factor acts on megakaryocyte precursors by stimulation of cytokinesis on the 2c level (megakaryocyte colony stimulating factor, Meg CSF), thus leading to an increased influx of 2c megakaryocyte precursors into the megakaryocyte compartment. The other, thrombocytopenia stimulating factor (TSF) – which need not be restricted to thrombopoietin since it can be derived from several sources – stimulates polyploidization and cytoplasmic maturation of megakaryocytes [46, 52, 151, 187].

Auxiliary Bone Marrow Cells and Lymphokines

In the past 2 years the observation of Williams et al. [189] that auxiliary bone marrow cells are required for optimal murine megakaryocyte colony formation has been supported by several partially contradictory studies.

Geissler et al. [48] has demonstrated that activated T lymphocytes from bone marrow of healthy volunteers augmented proliferation of human bone marrow megakaryocyte colony forming units (CFU-Meg). Kanz et al. [73] also demonstrated that T₄ lymphocytes, and to some degree T₈ lymphocytes, are important sources of stimulation factors needed for proliferation and differentiation of megakaryocyte progenitor cells. Gewirtz et al. [53], however, found that in the absence of endogenous stimulating factors only NK cells significantly affect normal megakaryocyte colony formation in vitro.

Quesenberry et al. [150] could show that two separate helper T-cell derived lymphokines, interleukin 3 (IL-3) and GM-CSA-2, were found to stimulate in vitro megakaryocyte colony formation. Since interleukin 3 was purified from WEHI-3-conditioned media, it was concluded that IL-3 appears to be at least one Meg CSF present in WEHI-3-conditioned media [172].

The two-factor hypothesis on megakaryocytopoiesis has recently been challenged by Burstein [22], who found evidence that IL-3 does not act only on the level of megakaryocyte progenitor cells but also promotes the maturation of murine megakaryocytes in vitro. In addition, it has been reported that cholinergic substances such as carbamylcholine enhance megakaryocyte colony growth [24]. Gamba-Vitalo et al. [44] and Chatelain et al. [27] observed a stimulating effect of lithium on CFU-Meg. The enhancement of colony formation by lithium is probably due to stimulation of T lymphocytes [27].

Much research is currently being done on the regulation of megakaryocytopoiesis, above all in tumor disease, and new approaches for understanding and therapy of thrombocytopathic diseases are to be expected from these studies.

Polyploidy of Megakaryocytes in Mammals and in Human Diseases

It follows from the results of the studies summarized that the degree of megakaryocyte polyploidy is undoubtedly controlled by thrombopoietin or thrombopoietin-like activities. Since the first DNA measurements of megakaryocytes in rabbits and

guinea-pigs [45, 86], the polyploidy distribution pattern of human megakaryocytes has been measured by several authors [83, 87, 147, 192]. It is apparently similar in all mammals investigated [132, 151], although measurements until now have only been carried out in mice, rats, guinea-pigs, rabbits, monkeys, and man. According to these investigations two-thirds of bone marrow megakaryocytes belong to the 16c class and approximately one-sixth to the 8c and the 32c class; 64c nuclei are rare in normal bone marrow of healthy mammals. In flow cytometric measurements Levine et al. [94] found a higher amount of 8c megakaryocytes, which was presumably caused by the enrichment procedures [151]. Jackson et al. [69] investigated murine marrow megakaryocytes with flow cytometric techniques but without previous enrichment, and also found a predominance of 16c nuclei.

Myeloproliferative Disorders and Leukemias

Measurements of megakaryocyte polyploidy in human diseases are still rare and have been performed only on a very small number of patients. Penington [131], Queisser et al. [149], and Lagerlöf [83] studied a total of 17 patients with myeloproliferative disorders. The results were inconsistent because patients in different stages of the diseases were investigated. Nevertheless, in polycythemia vera and megakaryocytic myelosis, a shift to higher polyploidy values occurs, whereas in chronic granulocytic leukemia megakaryocytes with lower DNA values (4c, 8c) predominate.

Since malignant transformation occurs at the level of the pluripotent stem cell in myeloproliferative disorders, megakaryocytes are very likely to be involved in the malignant process. In myeloproliferative disorders the megakaryocytes actually display an altered size, with micromegakaryocytes and bizarre-shaped nuclei [50, 152]. Queisser et al. [148] studied megakaryocytes of 11 patients with acute leukemia and preleukemia, and found an impairment of the labeling index with tritiated thymidine combined with a decrease of polyploidization which was reversible after remission.

Idiopathic Thrombocytopenic Purpura (ITP)

There are contradictory results concerning megakaryocyte polyploidy in patients with idiopathic thrombocytopenic purpura (ITP). Measuring five patients with ITP, Kinet-Denoel et al. [79] described an increase in megakaryocytes of lower polyploidies, whereas Penington [131] reported a shift

to higher polyploidy values in three patients with ITP. Nomura et al. [116, 117] and Queisser et al. [147] observed no alterations in polyploidy patterns of megakaryocytes compared to controls. In a study of the clinical course of 105 patients with confirmed chronic ITP (adult form), measurements of megakaryocyte polyploidy of 49 patients revealed that polyploidy distribution patterns vary on a large scale between low, normal, and high polyploidy. These different polyploidy values have no prognostic value. There is, however, a negative correlation between polyploidy and cytoplasmic maturity, as well as polyploidy and the peripheral platelet count. The lower the platelet count, the more immature the megakaryocytes and the higher the polyploidy of these cells [193]. This fits with the experimental results in thrombocytopenic animals mentioned above.

Uremia and Liver Cirrhosis

A similar observation was made studying the effect of uremia on megakaryocytes in 20 patients with end-stage renal failure. However, it could be demonstrated that high creatinine and blood urea nitrogen levels as well as hemoglobin and creatinine clearance values correlated with low average polyploidy of megakaryocytes [195], which was interpreted as a cytotoxic effect of uremia. In another study of patients with liver cirrhosis, a strong correlation between the amount of immature bone marrow megakaryocytes and the degree of thrombocytopenia could also be seen [191]. At the same time, a shift to higher polyploidy values could be demonstrated in nearly all these patients. For the sake of completeness, it should be mentioned that Queisser et al. [147] and Meyer et al. [100] investigated three patients with pernicious anemia and three patients with May-Hegglin anomaly, and found a normal polyploidy distribution pattern of bone marrow megakaryocytes.

Polyploidy of Megakaryocytes in Patients with Malignant Tumors

Investigations on a larger number of patients with pathologically proven diseases have become possible since the use of bone marrow obtained up to 12 h post mortem has become feasible [192]. In an extensive study, megakaryocyte polyploidy of more than 80 patients with malignant tumors with and without paraneoplastic thromboses was measured [192, 194]. All patients with metastatic malignomas displayed a significant shift to higher ploidy values irrespective of whether they suffered from

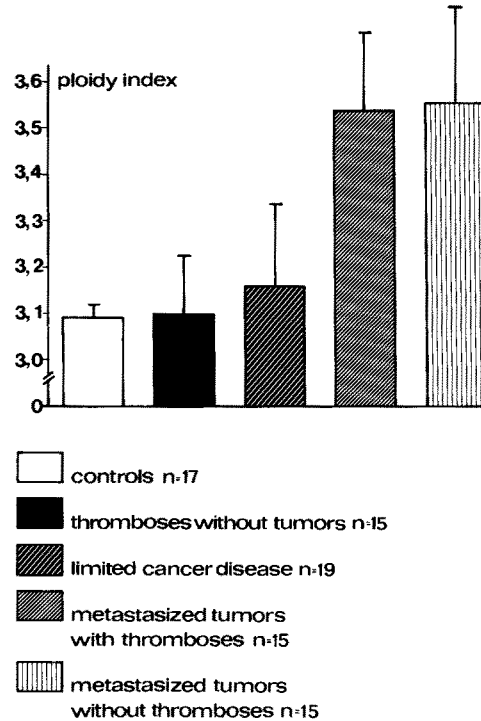


Fig. 4. Megakaryocyte ploidy indices of controls and of different groups of patients. Ploidy index is the sum of DNA replications (i.e., 8c megakaryocyte = 2 DNA replications) of all megakaryocytes measured divided by the total number of megakaryocytes measured per patient

thrombotic complications. However, patients with localized cancer had no or only slight differences of their ploidy indices compared to controls, as did patients with autopsy-proven thromboses but without malignant tumors (Fig. 4). By assessing the tumor volume of 38 patients with localized and metastatic tumors, a significant correlation between polyploidy and exponential growth of the tumor mass could be demonstrated (Fig. 5).

The Significance and Consequences of Polyploidy

The significance of polyploid cells and tissues continues to be a matter of discussion and research. In man polyploidy seems to be the main mechanism by which more or less stable tissues compensate an increased functional demand. Those postmitotic tissues which lose part of their ability for proliferation (cytokinesis) respond to the removal of parts of their tissues by an increase in polyploidization (partial hepatectomy, partial nephrectomy, heart muscle after infarction). In rapidly proliferating tissues such as skin or intestinal mucosa polyploidy has not been observed except in tumors. Conversely, important polyploidy seems to occur mainly in highly differentiated tissues with high

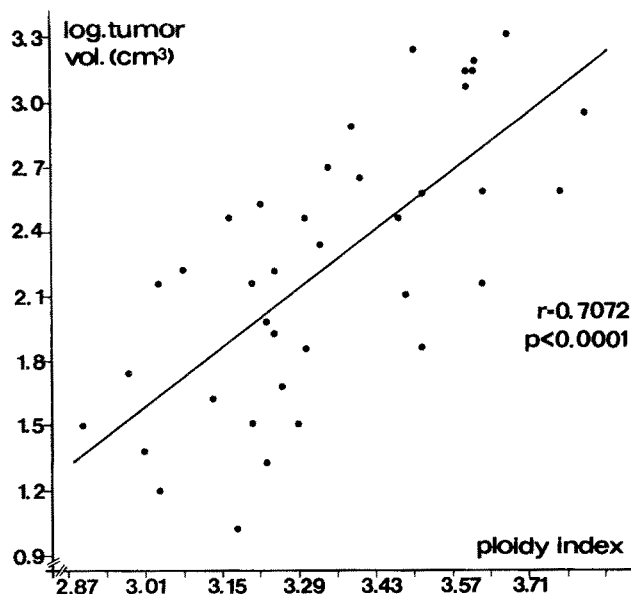


Fig. 5. Correlation of log tumor volume/megakaryocyte ploidy index in 38 tumor patients

synthetic activities. One hypothesis, therefore, is that an increase in synthetic activities finally leads to polyploidy. An increase, for instance, in protein synthesis is correlated with increasing m-RNA synthesis, which is somehow dependent on the amount of DNA present. In the case of an increased protein demand, an increase in DNA is therefore necessary to reach a certain level of protein production. This can, of course, be achieved by cell proliferation. However, some disadvantages are connected to mitotic cycles which the described endocycles do not possess. Cells undergoing cytokinesis are thought to be more sensitive to disturbances that would upset the complex events of their mitotic activity.

The major advantage of endocycles is that RNA or protein synthesis can go on during DNA replication. In *Allium carinatum* root tips it has been demonstrated that RNA synthesis continues during the endomitotic cycle but ceases during mitosis [112]. In the studies made by Nagl [112] it has further been demonstrated that capacity for RNA synthesis is higher in the endo- G_1 phases of tetraploid nuclei compared to the G_2 phase of mitotic diploid nuclei. Therefore the advantage of polyploidy may be seen primarily in an increase of protein-synthesizing activity.

Why is the cell so extravagant? Why does it not replicate only those chromosome segments needed for synthesis of highly demanded special proteins? Is the rest of the genome only replicated because it is not independently controlled? An ex-

planation for this "waste" of apparently useless genetic material could be that an otherwise created aneuploidy, as seen in tumor cells, could initiate malignant growth by replication of protooncogenes without replicating their control genes. Another ability of polyploid cells is that differentiation persists while replicating DNA. In bone marrow megakaryocytes differentiation from the megakaryoblast to the promegakaryocyte occurs while DNA content is replicated. In contrast, in many diploid cells tissue-specific protein synthesis sharply replaces proliferative forms of synthesis when the cell drops out from the mitotic cycle and begins to differentiate – a cell either divides or differentiates.

From all this it seems possible that polyploidy is more a consequence than a cause of simultaneous differentiation and proliferation. This leads to another aspect of the genesis of polyploidy. Brodsky and Uryvaeva [19] assumed that the properties and metabolic resources of a cell are limited. Simultaneous ongoing of proliferative and tissue-specific functions leads consequently to a more economic process in DNA replication – the polyploidization. It requires less membrane synthesis and no chromosome condensation.

What Does Polyploidy Mean for Megakaryocytes?

The striking consequence of polyploidization is the enlargement of cells. In megakaryocytes cell volume and nuclear volume have been proven to be strongly correlated with nuclear DNA content [95]. The surface, however, increases only 1.59 times with each doubling of volume. Thus, polyploidy reduces the surface/volume ratio. This might have some metabolic advantages. At least the amount of plasma membrane material used is less in polyploid than in diploid cells with similar DNA content. This leads to the significance of polyploidy in megakaryocytes.

Megakaryocytes are somehow unique among polyploid mammal cells. On the precursor level they have not lost their proliferative activity independent of the mammal's age. Once having entered the incomplete mitotic cycle, they stop cytokinesis and develop into highly polyploid cells. Regarding the evolution of the hemostatic system, nucleated thrombocytes emerge with the development of a closed circulation system with higher blood pressures in lower vertebrates. Further development of circulation in mammals with high blood pressures demands highly effective hemostasis which cannot be done by a limited number of nucleated thrombocytes as in the more primitive vertebrate

forms. The development of the stem-cell-megakaryocyte-blood platelet system enormously amplifies the efficiency of this hemostatic system. By shedding cytoplasmic fragments, approximately 3000 hemostatic active cell fragments are set free by a 32c megakaryocyte compared with only 16 nucleated thrombocytes by mitotic division, although the amount of replicated DNA is equal [163]. In each cell under mitosis there is a stimulus for synthesizing plasma membranes which coat the developing two daughter cells. This stimulus of membrane production seems to be fully used in megakaryocytopoiesis, providing the demarcation membrane system and the surface-connected canalicular system. From the investigations of Penington et al. [135, 137, 138], it could be shown that the cytoplasm of higher polyploid megakaryocytes contains a higher amount of demarcation membrane system, while 8c megakaryocytes are endowed with a greater content of granules and mitochondria. Thus, it has been concluded that platelets of higher polyploid megakaryocytes contain more membrane substances and less granules and mitochondria and are therefore of lower density, while platelets of lower polyploid megakaryocytes reveal an opposite effect of cytoplasmic organelles and membranes [70]. The higher amount of membrane substances which are the source of arachidonic acid for the synthesis of prostaglandins is thought to have significant functional differences because of the different amount of platelet components.

Keeping in mind the shift to higher ploidy values in thrombocytopenia, the results of Martin et al. [98] are important in explaining the consequences of polyploidization of megakaryocytes. They could actually demonstrate an increased thromboxane B₂ production per unit volume of platelets produced after 24 h of thrombocytopenia. Therefore, it follows that platelet heterogeneity depends more on the different DNA content of the megakaryocytes than on aging, as assumed by Karpatskin [74, 75]. Alterations in megakaryocyte polyploidy distribution therefore determine alterations in platelet population, function, and thrombotic complications. In patients with metastatic tumors, such an altered platelet population could easily favor hemostatic complications such as thromboses, which are to be expected in more than 50% [4] of all patients with metastatic tumors. The reason for this significant shift to higher ploidy values of bone marrow megakaryocytes could be the direct production of thrombopoietin-like activities by the tumor. Another explanation could be the activation of the monocyte macrophage system

in metastatic disease followed by the production of different lymphokines.

Further investigations, however, are necessary to clarify the causes and the consequences of altered megakaryocyte polyploidy and platelet heterogeneity and their significance for hemostatic disorders in malignant tumors and other human diseases.

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