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Wound Healing Is a First Response in a Cancerous Pathway: Hyperplasia Developments to 4n Cell Cycling in Dysplasia Linked to Rb-Inactivation

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Abstract

In a series of publications, the hypothesis of a special-type of endo-polyploidy, marked by 4-chromatid chromosomes (diplochromosomes), in the initiation of tumorigenesis has been presented from *in vitro* experiments. This review uses cellular happenings in benign pre-neoplasia to substantiate this idea, which appears to be linked to the wound-healing process of injured tissue. Rarer association between a wound healing process and a cancer occurrence has long been known. The wound healing multi-program-system involved a phase of tetraploidy that showed diplochromosomes. The hypothesis is that the inflammatory phase may not always be sufficient in getting rid of dead and damaged cells (by apoptosis and autophagy), such that cells with genomic damage (DNA breakage) may survive by genomic repair associated with change to diplochromosomal tetraploidy. *In vitro* data have shown division of these cells to be an orderly, mechanistic two-step, meiotic-like system, resulting in only two types of progeny cells: 4n/4C/G1 and 2n/2C/G1 pseudo-diploid cells with hyperplastic-like growth-morphology. *In vivo* damage to tissues can be from many sources for example, physical, toxic environment or from a disease as in Barrett's esophagus (BE) with acid reflux into the esophagus. For this condition, it is acknowledged that damage of the esophagus lining is a pre-condition to hyperplastic lesions of pre-neoplasia. These initial lesions were from "diploid" propagating cells and, 4n cells with G2 genomic content (no mitosis) accumulated in these lesions before a change to dysplasia. Cell cycle kinetics put these 4n cells in G1, which with S-phase entry would lead to asymmetric tetraploid mitoses, characteristic for dysplastic lesions. This change in hyperplasia to dysplasia is the root-essential condition for a potential progression of pre-neoplasia to cancer. In BE the hyperplastic lesion showed increasing gains of cells with inactivated p53 and p16[ink4a] genes, which destroyed the retinoblastoma (Rb) protein-control over S-phase entry from G1. Rb-protein is a key controller of cycling advancement from G1 (also for normal cells), and is frequently inactivated in tumor cells. Thus in BE, 4n/4C/G1 cells with

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mutated p53 and p16[ink4a] genes gained cycling ability to tetraploid aneuploid cell cycles, which constituted the change from hyperplasia to dysplastic lesions. In general, such lesions have high predictive value for a cancerous change. Proliferation rates of pre-neoplasia and progression have been shown to be increased by a component of the wound healing program.

Keywords

Mitotic Slippage, Endotetraploidization, Diplochromosomes, Meiotic-Like Division, 4n/4C/G1 Progeny, Proliferative Advantage, Inactivated p53, p16[ink4a]

1. Introduction

A fundamental requirement of cancer cells is gain of a proliferative advantage (GPA) or as frequently expressed "gain in fitness" over cells of origin, which are normal human, diploid cells. The mutation theory (MT) says: "It is well accepted that virtually all cancers result from the accumulated mutations in genes that increase the fitness of tumor cells over that of the cells that surround it" [1]. This type of mutational change was proposed to derive from inherited, chromosomal instability (CIN), which were seen as the driving force of tumorigenesis. The actual mutational changes were suggested to occur in "CIN genes" [2] and eventually this progressing machinery for the creation of genotypic variant cells would lead to tumorigenesis. There is general agreement that tumorigenesis is a multi-stage development [3] for which, the present goal is to show from *in vitro* experiments and from *in vivo* biopsies that the beginning is from pre-neoplasia, which starts from hyperplasia lesions [4]. The development to "mature" pre-neoplasia with change to high, cancer-risk dysplasia involves very specific cellular happenings that in the tumorous end can lead to tetraploid aneuploid abnormal cell cycles in the trip-tetraploid chromosomal range [5]-[7]. Albertson; Heim.

One simple question is how cells with random mutations consistently in different patients give rise to beginning hyperplastic growth in pre-neoplasia, showing similar, specific growth patterns with morphological change to rounded cells, and in tumorigenic growth show loss of differentiated characteristics to embryonic-like cells. Another acquirement is gradual gain of independent living of tumor-to-be cells from loss of organismal control [8]. This repeated pattern of changes in tumorigenesis assumedly is from *in vivo*, environmental-caused selection, but there are other suggestions for this consistently repeated pattern of changes in tumorigenesis (see below). One happening not much considered in cancer development is the wound healing process, which is a multi-phased program that preceded hyperplasia lesions in pre-neoplasia of Barrett's esophagus (BE). It is known that in rarer situations the healing process can take a cancerous pathway [9] [10], and by some scientists, cancer is seen as "—a wound that doesn't heal—", claimed in recent public announcements (MedicineNet). The wound healing program begins with hemolysis and inflammation and ends with growing fibroblasts in scar-formation (Wikipedia), but to what extent it plays roles in tumorigenesis is poorly known.

From the above, it is apparent that the tumorigenic process is very complex, and not just a result from a mutational machinery associated with selection of fitness-increased cells. Molecular reductionist analyses for "key" cancer-mutations can most likely never deliver a molecular comprehending, wholeness of the tumorigenic process. Tumor beginning to three dimensional (3-D) spheroids in its completeness with vascularization, ability for metastasis and formation of immortal cancer stem cells (etc.) has been proposed to be a return to embryonic-like developmental programs, (associated with germinal-cell-immortality) from evolutionary conserved ontogeny (e.g., tumors as a blastula stage) [11] [12]. Interestingly, after scrutiny of hundreds of references the conclusion was that this idea "—do not explain the mechanism that drive the normal cell to be transformed into cancer cell—" [12].

The present approach to the tumorigenic process also involved a concept of evolutionary conservation, which however, was arrived at from a genomic-behavioral analysis of primitive unicellular organisms when meiosis was non-existent [13] [14]. In the present review, it is accepted that development to "mature" pre-neoplasia with dysplastic lesions is pre-programed from evolutionary conserved primitive ways for maintenance of procreative-integrity. Wrong replication of genomes to diploidy (or higher) for simple, haploid organisms had to be reduced, and non-disjunction was not the answer [15]. This review is just in time, for a new "conceptual paradigm"

of the cancerous process, suggested to be lacking in present molecular approaches to cure-type cancer therapy and prevention [16]. Paradigm means example/model, and this is in fact what is revealed by the present focus on cellular happenings in the development of the pre-neoplastic phase. A century ago cancer was suggested to originate from random injury to cells [8]. Today this suggestion is seen as injury to normal tissue, which would elicit a pre-programmed, complex, multi-staged wound-healing process (Wikipedia). The main question herein is how a wound healing process can go awry to a cancerous pathway, and in so doing, is this a rarer event or a general route to cancers?

1.1. Pre-Neoplasia: Historically Missed or Not Believed to Lead to a Cancerous Process

The genesis-pathway to pre-neoplasia and further to malignant cancer has not had the interest from cancer research as it deserves. Perhaps this void is understandable, because historically, in the histo-cyto- and clinical pathological fields, there were great debates over a reality of a cancer connected benign phase, but today the doctor has won: pathological judged benign, pre-neoplastic growths are being surgically removed. The present idea is that a "mature" pre-cancer is a necessary stage for advancement to cancerous growth in progression. As mentioned the initiating lesion is hyperplasia, which consistently show proliferation from pseudo-diploid cells, but their origin has not been explained. Applicable to this *in vivo* question, is very likely, the *in vitro* well characterized genome reductive mechanistic division of tetraploid diplo-chromosomes, observed in pre-senescence associated with "genome damaged", short telomeres [17]-[22]. This irregular division, being meiotic-like, gave rise to only two types of progeny cells, 4n/4C/G1 and 2n/2C/G1, with the latter cells being pseudo-diploid with hyperplastic-like growth-morphology [13] [14]. For a construction of a sequence of cellular events in pre-neoplasia, these *in vitro* experimental data and cellular happenings in pre-neoplasia especially from ulcerative colitis and Barrett' esophagus (BE) were mainly considered. An important issue within this framework is whether pre-neoplasia in different organs (different tissue type and architecture) share common cellular events?

1.2. Tissue Damage, Wound Healing and Cancer

An early experiment with non-carcinogenic cover-slip glass pieces (foreign body response) implanted under the skin of rodents, elegantly in its simplicity, demonstrate initiation to pre-neoplastic growth from a beginning induction of genomic damage [23]. In these experiments the so-called "transformed" cell-growth (a rather confusing term in tumorigenesis) appeared first on the edges of the glass pieces. The question is why on the edges? Because, skin cells plus other types of cells (stromal) would be physically injured with a "tissue" elicited wound-healing response. Wound-healing as mentioned, is a complex program with different phases, which in rarer situations during a tetraploid phase, showed tetraploidization to 4-chromatid chromosomes (diplochromosomes) in metaphase. This unusual happening was suggested to show a wound-healing that had gone wrong into a cancerous process [9] [10]. In the cover-glass experiments the sequence of wound healing with a polyploid-phase also likely happened with the appearance of "transformed cells". Recently, bone cancers, claimed to be injury related in young children were evaluated [24]. The fact that their bones were still growing appeared to be a strong risk factor for advancement to cancer (sarcoma), which agrees with the fact that cancer in general, occurs in tissues that are growing (e.g., re-placement growth in intestine and skin). The wound-healing process, starts with bleeding (hemolysis) and an inflammatory phase, which removes dead and damaged cells, broken down to debris by apoptosis and autophagy (Wikipedia). The hypothesis for a wound healing program in cancer development is herein suggested to come from an incomplete clearance of genome damaged cells. Such cells could turn to DNA repair ($^{\gamma}$H2AX-foci), which would have probability of special type tetraploidization with diplochromosomes through mitotic slippage (see below).

1.3. Mitotic Slippage in the Origin of Tetraploid Diplochromosomes

Tetraploidy showing diplochromosomes in pre-malignant cervical lesions led to the question of the origin of the 4-chromatid structure [25] [26]. Known is that mitotic slippage occurs for G2 arrested, bichromatid chromosomes (normal metaphase chromosomes) undergoing a prolonged genome repair process [27]. For such G2-repair, the mitotic entry protein, cyclin-B-cdk1-kinase degenerates such that the metaphase-type chromosomes repaired or not, skips mitosis and goes directly into S-phase. There they undergo an extra genome-wide loading of cohesin [28]-[30] while the DNA double helix of each chromatid "unwinds" and replicate to two chromatids

giving rise to 4-chromatid diplochromosomes [18] [19]. These authors verified the diplochromosomal structure, but missed their unusual orderly, genome reductive divisions (meiotic-like) back to diploidy [9] [20]-[22].

From the above Uhlman [31] in consideration of evolutionary conserved genome-wide extra loading of cohesin, concluded that it was an: "—integral part of the response to genomic damage—". In other words diplochromosomal construction is triggered by cells in G2, undergoing genomic repair that takes a long time for resolution, such that mitotic entry molecules disintegrate. Importantly, during the replication process the centromere region between the two chromatids stayed cohesed, and became under-replicated [32]. Chromosomal instability (CIN) was evident in subsequent genome reduced pseudo-diploid propagation (breakage and laggards) [33]. Hopefully, this out-line of the formation of 4n/8C, diplochromosomes becomes helpful in an acceptance of their important mechanistic reductive division-system, to two types of progeny cells: 4n/4C/G1 and pseudo-diploid 2n/2C/G1. These latter cells in G1 is expected, but 4n cells with G2 DNA content in G1 is a paradox (see below).

1.4. How Can Tetraploid Diplochromosomal Cells Genome-Reduce in an Orderly, Mechanistic Way?

Forty-six four-chromatid chromosomes on a spindle apparatus evolutionary evolved for 46, 2-chromatid chromosomes, predicts everything else, but an orderly segregation, but as already described, such is accomplished by the mechanistic genome reductive division. Recently, it was reported that chemo-associated tetraploidy returned back to diploidy and caused tumor-relapse [34]. Several comments on this unknown happening in cancer, discussed how there could be orderly segregation to diploidy from tetraploidy [35] [36]. Unaware (assumed) of existence of diplochromosomes in mouse ascites cancer [37], and in the prototype cancer model, Bloom's syndrome [38] [39], the tetraploid condition was assumed to be from the simple genomic doubling to 92, two-chromatid chromosomes. A calculation for probability of a return back to genome balanced diploidy from random distribution of 92 chromosomes in mitosis was at best one cell among 6×10^{12} segregation-products [35]. This calculation demonstrated that the orderly, tetraploid return to diploid cells was achieved by something special, which for *in vitro* diplochromosomal cells is co-segregation of whole genomic complements [12] [33]. But the winning argument was somatic genome reduction as known from the mosquito [36]. Interestingly, co-segregation of whole genomes is a feature in reductive divisions of mammalian trophoblastic cells [40]. In primitive unicellular haploid organisms, reduction with co-segregation from diploidy for return to haploidy was accomplished by the chromosomes forming a ring from end-to-end attachments followed by ring separations in division [41]. Now and then, there are claims of cancer cells showing presence of chromosomal end-to-end associations, which could not be explained by telomere-associations.

Unfortunately, references to reductive divisions in the gut of the *mosquito*, and other dipterans (male haploidy), provokingly, is based on plain ignorance. The mosquito belong to the *Diptera* Class, which has evolutionary special, evolved capacity for somatic pairing of homologous chromosomes. This was historically, shown by Curt Stern [42] who demonstrated somatic crossing over in *Drosophila melanogaster*, which require pairing of homologs. Thus, referrals to the mosquito meiotic-type reductive divisions, must stop, and are not valid explanations/-interpretations for the *in vivo* tetraploid-associated reductive division back to diploid cells, more resistant to original therapy treatment [34]. Diplochromosomes are not meiotic-like "tetrads" as indicated by some scientists, and higher level endopolyploidy in human cells do *not* fragment amitotically to lower ploidy-levels, because of inborn *mosquito-like* meiotic-type, genomic reduction.

1.5. A Carcinogen-Free Induction System for Induction of Diplochromosomal Cells

In flask cultures, 5 - 7 weeks in nutritionally supported senescence (induced by dysfunctional telomeres) there appeared three-dimensional (3-D) tumor-like spheroids with dysplastic-like growth from cell polarity changed cells [43]. This occurrence led to the question of whether this would happen for young, normal cells with long, normally-behaving telomeres. Therefore, a carcinogen-free induction system was needed, and metabolic studies of amino acid deficiency (AAD) had shown endo-replicated cells [44]. Accordingly, young human primary cells in flask cultures were exposed 2 - 3 days to medium deficient in amino acid glutamine, and serum was reduced to 2% (M−) (repeated several times and with 4 - 5 days in M−). This was followed by 1 day in normal medium (M+) for beginning, recuperation to dividing cells. Next day, aliquots of cells (3 - 4000) were seeded onto chamber slides, which were harvested at day's intervals for *in situ* observations of resulting cell proliferations.

The choice of deficiency for glutamine was based on it being an important source for nitrogen and carbon for protein synthesis (nucleotides in DNA repair) [45] [46]. Lack of dietary amino acids, specifically glutamine, contribute to deadly inflammatory bowel disease in the world [47]. In these experiments the genomic damage was from stopped replication forks [48] [49], which showed repair by exchange figures in meta- and anaphase with relatively high segregation rate of centromere broken chromosomes. The first mitoses in the recovery growth, and only then, were dividing diplo-chromosomal cells [33]. In passage growth, 3-D hyperplastic growth with dysplastic-like morphology from cell polarity change/loss were observed with seemingly random occurrences [50]. Thus, these special cellular happenings were not linked to the telomere-shortening phenomenon.

1.6. *In Vivo* Pre-Cancers: Hyperplasia

Hyperplasia being diploid is assumed to give rise to dysplasia with cycling tetraploid aneuploid cells, but how this occurs is unclear. Hyperplasia are of two types, typical and atypical, distinguished from normal growth with either uniform (metaplastic), streaming, swirling, growth patterns or similar, but more variable with cyto-nuclear pleomorphism [4] [51]. The atypical "—has long been recognized as a frequent preliminary morphologic change of human tumors" says pathologist Bignold [52]. Dysplasia, defined as "loss of normal cell orientation" [53], express dis-ordered growth patterns from cell polarity change/loss, cell/nuclear-morphology changes, with/without hyper-chromatism, and importantly, distorted nuclear to cytoplasmic ratios. Noteworthy, the two phases, hyperplasia and dysplasia are pathologically treated differently, in that dysplasia is graded from mild to medium to severe, suggesting a gradual increasing abnormal growth, whereas there are no grading system for either hyperplasia. This fact in itself is remarkable, because it suggests that something abruptly happens for the transition/conversion from hyperplastic to dysplastic cells.

As mentioned, pre-neoplasia in ulcerative colitis and Barrett's esophagus (BE) disease were the main contributors [53] [54]. The molecular complexity of BE disease-initiation and advancement to adenocarcinoma is outside the scope for this discussion. However, it is noted that the route to BE hyperplastic lesions is from repeated, stomach acid reflux into the esophagus, which causes injury to the normal esophagus tissue. This would provoke a wound-healing process, and inflammation would rid the damaged tissue of dead and injured cells. However, as mentioned this clearance may be incomplete, and DNA repair of the "left-behind" genome damaged cells can lead to the special type tetraploidy with diplochromosomes in BE (see mitotic slippage). Their division would lead to the two types of progeny cells, 4n/4C/G1, and the 2n/2C/G1 cells that *in vitro* showed hyperplastic-like pseudo-diploid growth. The question is whether this would be a rarer event? Not likely, in fact the "rarer occurrence" of a wound healing-associated cancer development is likely a misnomer. Because in these rarer cases, the time of cancer occurrence was short and could be traced back to time of injury, whereas cancers in general take 10 - 20 years for development, and a correlation to time of injury would be impossible. The wound healing system has been shown to influence metastasis events [55], and also tumor angiogenesis [56].

1.7. 4n Cells in Hyperplasia Lesions

The next question is, what are the pathological cellular happenings in the pseudo-diploid hyperplasia that lead to the origin of dysplastic lesions? Pathologist Steinbeck [53] made special issue of endo-re-replication (mitotic slippage) of cells in pre-neoplasia of ulcerative colitis versus continued replicating endocycles (in differentiation). He noted that 4n cells accumulated before presence of what he called *pathological mitosis*, which by photometric DNA measurements of individual cells (division figures) showed asymmetric (aneuploidy) cycling of tetraploid cells in the 5C extended range. This important event, accumulation of 4n cells in hyperplasia lesions was also observed in conjunction with p53 inactivation in BE [57] [58]. Fluorescent *in situ* hybridization (FISH) of BE pre-neoplastic hyperplastic lesions, showed that the cytometric 4n fraction (verified by centromeric FISH probes) showed "4N (G2-polyploid)" DNA content. But, although these 4n cells in G2 were expected to go into mitosis they did not divide, which was assumed to be caused by arrest from a G2 checkpoint control [57]-[62]. A G2 checkpoint arrest was also claimed for similar 4n cells with G2/M DNA-content for early "precursor lesions" of several different cancers [63].

The paradox with these observations was that these 4n cells with DNA-content of G2 were not in G2, but in G1. Such was actually indicated from studies of *in vitro* cultivated BE pre-neoplasia, biopsy material [64]. Support for this view also comes from another cytometric analysis in pre-senescence where diplochromosomal cells [18] [19] from cell cycle kinetics divided into two 4n/4C daughter cells in G1, the conclusion was: "G2/M (4n)

DNA content represents G1 tetraploid cells" [65]. This problem of G1 or G2 placement of 4n/4C cells has very likely hindered a much earlier conclusion from observed events in BE, which would lead into high risk dysplasia, a known forerunner for change to adenocarcinoma. Although, high risk value was predicted from "2.7N" DNA value and not below [58], the question of dysplasia-origin from pseudo-diploid hyperplastic cells was not answered. Neither was the origin of the accumulating "4N (G2 polyploid)" cells. In this report the 4n/4C cells are in *G1* from cell cycle kinetics from diplochromosomal, 4n/8C/M division to 4n/4C/G1 daughter cells. At this time in our knowledge there are no other division-mechanism known that can produce 4n/4C/G1 cells. These latter cells, if they can enter S-phase, there is probability of dysplastic, pathological mitoses giving rise to tetraploid-triploid karyotypes for some solid tumors (aneuploidy) [6] [7]. The information on the origin of dysplastic lesions is crucial for a fundamental concept of pre-neoplasia as the beginning of progression that can lead to malignant tumors.

1.8. Inactivated Genes with High Selective Value in BE Pre-Neoplasia

Change to dysplasia from the question of how 4n/4C/G1cells can gain entry into an S-period is connected to a type of molecular analysis that identify gene mutations more frequent than others. Such an investigation was done on a large scale for breast and colorectal cancers where frequencies of different gene-changes were placed into hills and mountains, with the hills seen as driving the tumorigenic progression [66]. For the more frequent mutated genes in the mountains, consisting of inactivated p53, APC and K-RAS, strangely, there were no suggestion of tumorigenic-function. Another way of gaining similar knowledge is from patients showing hyperplastic lesions with preferential inactivated genes, as for example, over 70% of patients with urothelial hyperplasia were positive for deletions on chromosome #9 (regions, arms or whole #9) [67]. This chromosome carries p16[ink4a] and CDK12 tumor suppressor genes on 9p [67] [68]. In pre-neoplasia of ulcerative colitis, abnormal chromosome arm-counts from loss was a cancer risk factor predictor [69].

These high frequency inactivated genes have led to the question of the selective pressure for these happenings in the tumorigenic process [70] [71]. For example, Gorgoulis and co-workers [71] looked for p53 mutations in non-small cell lung diploid hyperplastic pre-neoplasia and in the associated carcinomas, and also in pre-neoplastic nevi from different patients. They found that all hyperplastic lesions were negative for this mutation, whereas dysplasia and the cancers were positive (the latter yes and no). These data established that p53 mutational change was a late event for these pre-cancers, and that mutated p53 was not involved in the initiation-process to pseudo-diploid hyperplasia. But, in these lesions with normal p53 structure, genomic damage was demonstrated by presence of stained nuclear repair foci from phosphorylated histone H2AX ($^{\gamma}$H2AX) and other repair proteins. This genome damage-associated hyperplasia was experimentally verified by experimental induction (virus-vectors) of genomic damage with a resulting hyperplastic growth (GPA) from normal human cells. Genome damage (injured tissues) is one required precursor activity for activation of the evolutionary conserved "relic" DNA, which determines reductive mechanistic divisions [13] [14]. Interestingly, in the various proposals for a cancer initiating mechanism, there is little to no considerations of evolutionary conserved cellular phenomena.

1.9. Retinoblastoma Gene (Rb) in Hyperplasia

In BE a high percentage (60% - 70%) of patients presented with hyperplastic lesions that contained inactivated tumor suppressor genes, p53 and p16[ink4a] [70]. Inactive p16[ink4a] occurred early (see above #9), and preceded p53 (on 17p) [72]. Thus, in BE pre-neoplasia these particular genes "experienced" high selective pressures, and the question is why? Known is that the hyperplastic lesions contain accumulated 4n/4C cells in G1 of the cell cycle, which can only re-enter the cell cycle from entry into S-periods linked to mitosis with CDKs low in G1 and high in S-G2-M [[73] [74], Heng]. For normal diploid cell-cycling from G1 to S, there is a "restriction point" in late G1, where the retinoblastoma (Rb) protein is the main controller of S-phase or G0 entry [70]. Thus, for 4n/4C/G1 cells to enter S-phase, Rb control must be inactivated. Loss of Rb function have been shown to be very frequent in cancers, and experimentally has shown a variety of abnormal proliferative happenings [75] [76]. In BE the facts were that the Rb gene was structurally normal, but that the inactivated p53 and p16[ink4a] genes played roles in the inactivation-process of the Rb-protein [70]. Thus, with loss of Rb control over S-phase-entry, the gate would be open for 4n/4C (-/-) p53, (-/-) p16[ink4a] cells in G1, to start replication to 4n/8C/G2 cells for asymmetric, aneuploid mitoses, which constituted a change to dysplasia. Similar loss of Rb-function for quiescent cells resulted in immediate, cell cycle entry [77]. Davoli and de Lange [19] pointed out that near-tetraploid

karyotypes were positively correlated to frequency of inactivated Rb, and suggested: "—an important (but unexpected) role for Rb loss in tetraploidization during tumorigenesis—". Logically, if Rb-loss can result in cycling ability of 4n/G1 cells with mutated p53 and p16[ink4a], it is expected that these mutations in the hyperplastic diploid cells would also affect their proliferative ability. Thus, the positive selection pressure for mutated p53 and p16[ink4a] in BE is linked to Rb-inactivation. The asymmetric divisions (pathological mitosis) would not only give rise to cells in the extended 5C range, but their sister cells would have much lower chromosome numbers, which agrees with the fact that the karyotypic chromosome number for different solid tumor-types can be below 46 and up-ward [6]. The continued proliferation in progression from dysplasia depends on selection of progeny cells, endowed with genetic and epigenetic changes resulting in increased fitness. A new finding is that the inflammatory process of the wound healing system "exacerbates" the growth in pre-neoplasia and progression [78]. An interesting suggestion has come from the fact that BE pre-neoplasia and matched adenocarcinoma shared pre-cursor molecular markers [79], a commentary suggested that "—genetic determinants of progression —" were "—preprogrammed from a very early stage in each potential cancer's life—" [80]. Is this idea in line with the discussed ontogenetic, evolutionary conserved "genetic" preprograming of the cancerous process [11]-[14]?

This new scenario of cellular events leading to the critical dysplastic phase in tumorigenesis can be tested in cell cultures by use of the present carcinogen-free induction, which would produce 4n/4C/G1 cells from diplochromosomal tetraploidy. Following flow cytometric isolation, and manipulations to inactivate constitutional alleles of p53 and p16[ink4a] for Rb-inactivation, the question is: will these 4n cells start aneuploid, asymmetric proliferation? If the answer is positive, it could mean new targets in cancer therapy treatment and prevention. It would also be a significant step forward in cancer research with probability of cancer-incidence reduction, perhaps eventually via vaccination.

2. Conclusion

Cancer is known to be a rarer occurrence in a wound-healing process of injured tissue. It is a very complex multistage, program starting with hemolysis and an inflammatory response. When the inflammatory response for the damaged tissue is insufficient in removing debris from digested dead and injured cells, there might be present cells with genomic damage capable of turning to survival mechanisms. A number one event would be DNA-repair and endo-tetraploidization to diplochromosomes that can undergo reductive division to two types of offspring cells: 4n/4C/G1 and 2n/2C/G1. In pre-neoplasia of ulcerative colitis and Barrett's esophagus, these two types of cells were instrumental in revealing a rather complex route from hyperplasia to dysplasia. Dysplasia growth with tetraploid aneuploid cell cycles is well known to be a high risk factor for change to cancer. This means that for the first time the cellular step to the critical beginning of dysplasia has been identified, which gives probability of negating such development with result of cancer incidence reduction. The basic question was how in hyperplasia of pre-neoplasia 4n/4C/G1 cells could enter the cell cycle, which was solved by destruction of Rb-protein-control over S-phase-entry. This was accomplished for BE by selective presence of inactivated p53 and p16[ink4a] genes. When these mutated genes were present in the 4n/G1 cells, they destroyed Rb-protein control over entry into S-phase. Thus, with freedom to replicate to 4n/8C cells, the potential for tetraploid aneuploid cell cycles was present. Importantly, this change constituted a beginning of dysplasia in hyperplasia lesions, and pre-neoplasia was "mature" for a change to progression. Today, the genomic damage from the mutation theory is the focal point in the "war on cancer", which now is challenged by the present factual, cellular happenings in the origin of dysplasia, a pre-cancerous condition in tumorigenesis. The future will most likely uncover other high frequency selected mutated genes associated with Rb-inactivation, which is already known for breast and colon cancers (p53, APC, K-RAS), but without known functions in tumorigenesis.

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