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Review

Chromothripsis and epigenomics complete causality criteria for cannabis- and addiction-connected carcinogenicity, congenital toxicity and heritable genotoxicity

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ABSTRACT

The recent demonstration that massive scale chromosomal shattering or pulverization can occur abruptly due to errors induced by interference with the microtubule machinery of the mitotic spindle followed by haphazard chromosomal annealing, together with sophisticated insights from epigenetics, provide profound mechanistic insights into some of the most perplexing classical observations of addiction medicine, including cancerogenesis, the younger and aggressive onset of addiction-related carcinogenesis, the heritability of addictive neurocircuitry and cancers, and foetal malformations. Tetrahydrocannabinol (THC) and other addictive agents have been shown to inhibit tubulin polymerization which perturbs the formation and function of the microtubules of the mitotic spindle. This disruption of the mitotic machinery perturbs proper chromosomal segregation during anaphase and causes micronucleus formation which is the primary locus and cause of the chromosomal pulverization of chromothripsis and downstream genotoxic events including oncogene induction and tumour suppressor silencing. Moreover the complementation of multiple positive cannabis-cancer epidemiological studies, and replicated dose-response relationships with established mechanisms fulfils causal criteria. This information is also consistent with data showing acceleration of the aging process by drugs of addiction including alcohol, tobacco, cannabis, stimulants and opioids. THC shows a non-linear sigmoidal dose-response relationship in multiple pertinent *in vitro* and preclinical genotoxicity assays, and in this respect is similar to the serious major human mutagen thalidomide. Rising community exposure, tissue storage of cannabinoids, and increasingly potent phytocannabinoid sources, suggests that the threshold mutagenic dose for cancerogenesis will increasingly be crossed beyond the developing world, and raise transgenerational transmission of teratogenicity as an increasing concern.

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1. Introduction to seminal paper

In a remarkable and highly celebrated report, the Pellman lab recently showed that severe chromosomal fragmentation involving dozens of double stranded breaks and subsequent apparently random and disordered repair of some of the fragments, could rapidly occur during the DNA synthetic phase (G2 and S-phases) of the mitotic cell cycle, if chromosomes became isolated from the main nuclear mass [1]. In this technical tour de force, high resolution DNA sequencing of single cells and live cell imaging was deployed to show that chromosomes which had become detached from the mitotic spindle or chromosomes became isolated in micronuclei, where, lacking the normal full complement of replication and repair enzymes, the DNA became shattered in the process of disordered and dysregulated replication. Such damage could become propagated through subsequent rounds of cell division, where the isolated chromosomes could also become joined up with those of the main nucleus. Where two or a few chromosomes were trapped together, in such a micronucleus random exchange could occur between them. Chromosome "pulverization" was first described in 1967 due to experimental viral infection [2] (Figs. 1 and 2). The process has recently been named "chromothripsis" for chromosomal shattering at hundreds [3] or thousands [4] of loci; and a milder form was called "chromoplexy" (chromosomal tangles or braids, Fig. 3) [5]. Extraordinarily, this process was shown to proceed as rapidly as within 16 h [1].

This remarkable result immediately resolved a long standing paradox in cancer research as to how such dramatic event could arise when the normal fidelity of DNA replication occurs with an error (mutation) rate of only 10^{-8}, and the rate in germ stem cells is one hundred times lower. It also simultaneously provided an elegant mechanism for the high rate of micronuclei, chromosomal fragments and abnormal chromosomes (truncated arms, chain and ring chromosomes and double minute circles [6]) which are frequently seen in malignant tissues (Fig. 4)[7]. Tetraploidy itself has been shown to increase chromosomal instability, tolerance of mitotic errors and the multidrug resistance typical of transformed and tumour cells and even the anchorage-independent growth of non-transformed cells [7].

In addition to cancer, such chromothriptic events have also been shown in various congenital abnormality syndromes [8–14].

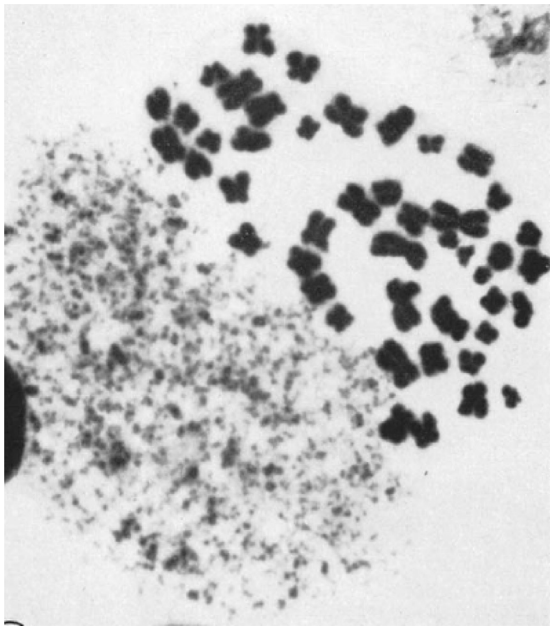

Fig. 1. Chromosomal Pulverization.
Original Report of Chromosomal Pulverization. Figure 7 , Kato H., Sandberg AA (1967). "Chromosome Pulverization in Human Binucleate Cells. Following Colcemid Treatment." J. Cell Biol. 34 (1): 35–45. Re-used by permission.

2. Dynamics of the cell cycle

The cell cycle has numerous check points which are designed to prevent such genetically catastrophic events from occurring. The mitotic spindle assembly checkpoint (SAC) in particular requires all chromosomes to be attached to the spindle, and sister replicates to be attached at their kinetochores with opposing polarity (bi-orientation) to bundles of microtubules of the mitotic spindle which will draw them to opposite poles of the cell [15]. Mostly errors in this complicated machinery [16–19] generate cell cycle arrest, apoptosis, or the irreversible entry into cellular senescence [7]. But delay at the SAC is not indefinite [15]. Some cells slip back as tetraploid cells into interphase and a very few escape cell cycle controls altogether. This can particularly occur when chromothriptic events involve the functional silencing of such major tumour suppressor genes as TP53 (P53) and CDKN2A (P16INK4A), which normally sense and amplify such cellular and senescence checkpoints [20]. Other genetic causes (mutations, insertions and deletions) also exist for tumour suppressor gene silencing. Hence the usual outcome of such events at the tissue level is; growth arrest via apoptosis, senescence or cell cycle delay [21], and occasionally malignant transformation where the malignant clone may have a growth advantage [7,22].

The pathway described by the Boston group [1] was therefore inhibition of spindle dynamics/failure of spindle attachment/micronuclear formation/chromosomal shattering or pulverization/haphazard chromosomal annealing by non-homologous end joining or microhomology-mediated break-induced replication, then cell cycle arrest or occasionally and alternatively, oncogenic transformation [3,12,20,22–25]. Chromothripsis has been described as occurring in about 2–3% of cancers including melanoma, sarcoma, lung, thyroid, oesophageal and renal cancers [4], although it is seen much more commonly in cancers of the bone (25%) [20,26], brain (39%) [27,28], bowel [29] and a majority of prostate tumours [5]. It has also been said to be more common in cancer per se, as the technical difficulties in unravelling the enormous complexities in sequencing errors to which it gives rise are only beginning to be probed [5,22,24,26,27,29,30]. Its presence and severity correlate with poor prognostic outcomes [27,30]. Progressive chromosomal instability instigated or assisted by chromothriptic and disorderly mitotic mechanisms also explain the usual tendency of tumours to become increasingly aggressive [26]. Curiously single cell chromothripsis has also been shown on occasion to cure rare genetic disorders [31].

The Boston work [1] also focussed attention on the extraordinarily complicated machinery associated with the microtubules comprising the mitotic spindle. Microtubules are primarily made up of α- and β- tubulin dimers which, together with their numer-