Mechanisms of Cannabinoid Genotoxicity

Subjects: Others

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The triple convergence of cannabinoid carcinogenesis, cannabinoid teratogenesis and the cannabinoid acceleration of aging together forms strong and theoretically robust evidence for a clinically and highly significant genotoxicity severe enough to impact numerous metrics of the population health adversely. Furthermore, both in vitro and clinical studies implicate many different cannabinoid moieties, suggesting that genotoxicity is a class effect shared by many cannabinoids—a feature now well confirmed by many epidemiological studies. This includes such allegedly benign cannabinoid species as Δ9THC, Δ8THC and cannabidiol, among several others.

tobacco alcohol cannabis

1. Fundamental Primacy of the Epigenomic Effects

1.1. Layers of Epigenomic Regulation

Many layers of epigenomic regulation are described and the list appears to be rapidly increasing. While they may be listed individually, they are not independent and are coordinated across the various layers. The key parameters include DNA methylation, the histone post-translational modifications, various short and long non-protein coding RNAs, over 100 post-transcriptional modifications to the RNAs, including m6-adenosine RNA methylation (also referred to as epitranscriptomics), the position with respect to the nuclear lamina (which is suppressive of the gene transcription), the chromatin state (euchromatin or suppressive heterochromatin), the presence within the transcriptional factories of the topologically defined domains (and their controlling boundary elements) and the presence of the tethering elements (especially important during the development) [1].

1.2. Epigenomic Functions

It is now well understood that the cell lineage specification (that is, whether a cell develops as a muscle cell or a neuron, etc.) is controlled epigenomically. This issue was first formalized in the epigenetic valley hypothesis of Conrad Waddington [2]. It is also well established that the state of the cell differentiation from a pluripotent embryonic cell to a mature fully differentiated cell is also controlled epigenomically.

For a long time, the mechanisms of aging were not understood and many competing and often complimentary and overlapping theories were advanced [3]. However recent studies have confirmed that, while there are many different pathways to induce age-related damage, the major controller of cellular age is actually the epigenomic state of the cell on which other pathways likely converge [4][5][6]. Thus, robust evidence of the reversal of epigenomic clock aging, biological age and the youthful/neonatal functional capacity has now been convincingly

demonstrated in many systems, including optic nerve crush injury, congenital glaucoma and ocular aging, progeroid mouse models, cardiac and skeletal muscle and fibroblasts [5][7][8][9]. This view is concordant with the well-established control of the state of the cell differentiation by the epigenomic machinery.

This implies that the epigenomic state is central and pivotal to the control of cancer, cell development and aging, which are the three principal themes of the present discussion.

2. Epigenomic Impacts of Cannabis Exposure and Withdrawal

A recent detailed epigenome-wide association study (EWAS) by Schrott and colleges investigating the DNA methylation changes of human and mouse sperm both in cannabis dependence and withdrawal provides a 359-page Supplementary Appendix listing the detailed methylation changes [10]. These researchers looked at the differential DNA methylation of the cannabis-dependent humans and mice compared to the cannabis free controls and again after an 11-week period of washout following a documented period of abstinence and detoxification from the cannabis. This longitudinal design is a very powerful way to design an epigenomic study. Close study of this dataset revealed the following remarkable findings.

2.1. Disruption of the Epigenetic Machinery

There was widespread disruption between the main readers, writers and erasers of the epigenetic code. Hence, there were five hits for the DNA methyltransferases which added the methylation mark to the CpG islands and one hit for TET1 (ten-eleven translocase) which began the process of removing it. There was one hit for telomerase which controlled the end length of the chromosomes, and thus protected them against aging, three hits for polycomb repressors, five hits for the chromatin remodelers (SMARCA's, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A) and three hits for the UHRF (Ubiquitin-like with PHD and ring finger domains) family which controlled both DNA methylation and histone methylation and integrated the signaling in the two classes of pathways.

There were 161 hits for the histone methyltransferases with methylate histones and 199 hits for the histone demethylases that remove this mark. There were eleven hits for both the histone acetyltransferases, which acetylate histone tailed and thereby made the genome accessible to the transcription machinery, and eleven hits for the deacetylases which removed this mark.

2.2. Stem Cell Renewal Factors

Considering the key stem cell induction factors identified by Yamanaka, Oct3/4, Sox2, Klf4 and Myc [7], all four were positively identified in this EWAS screen. When the EWAS screen was widened somewhat to include the other stem cell factors identified by the Yamanaka group and others [9], again many were positively identified, including Ras, catenins, Kit and the Lin28 microRNA.

2.3. Chromosomal Disorders

As noted above, chromosomal disorders hold a prominent place in the patterns of cannabinoid-related carcinogenic and teratogenic disorders. Indeed, when the length of all the chromosomes involved was summed (omitting duplications), it was found that an impressive 1765 megabases of the 3000 megabases of the human genome were directly implicated in the cannabinoid-related genotoxic disorders, which is 58.8% of the human genome. For this reason, the epigenomic findings of the Schrott EWAS dataset were of immense importance.

Additionally, the rays of the mitotic spindle were composed of microtubules of polymerized tubulin. There were 106 hits in the Schrott EWAS database for tubulins. Importantly, tubulin undergoes numerous post-translational modifications which are thought to govern its intracellular trafficking and organellar addressing [11][12][13]. The cannabis withdrawal disrupted the alpha tubulin acetyl transferase which was tasked with acetylating tubulin and thereby made the microtubules flexible and increased its tensile and torsional strength. This was important as the microtubules are normally bent during the spindle formation and tensioning. Failure of completing this action leads to microtubular breaks and fractures and, thus, the chromosomal derailment during the anaphase.

The centromeres are the critical central portions of chromosomes where binding occurs to the mitotic spindle. Centrosomal protein A (CENPA) was a modified version of histone 3 (H3) and CENPA replaced H3 in the centromere, which marked the location of the centromere. Upon this CENPA basis, a complicated scaffold of 17 proteins assembled which then was bound to the microtubules of the spindle via other kinetochore scaffolding proteins [14][15][16][17][18]. Fifteen different CENPs were identified in this EWAS screen, including 86 hits for CENPN, which was the second protein to bind to the centromere complex.

The proteins which cohere the ends of the human oocyte meiotic spindle so that two (and only two) spindle poles are formed, guiding the formation of two (and only two) daughter cells, are called centrosomal organizers. There were three hits for these proteins, including the nuclear mitotic apparatus protein (NUMA).

The motor proteins which actually move the chromosomes along the microtubules towards minus end of the microtubules and the spindle poles after the anaphase checkpoint is released are called dynein motors which are controlled by a binding partner known as dynactin. There were seven EWAS hits for dynein–dynactin. Interestingly the intracellular kinesin motor moved protein and other cargo in the opposite direction towards the positive end of the microtubule and 218 hits were recorded for kinesin motors.

Sumoylation was shown to be a key post-translational modification of the key proteins, which organizes the chromosomes and are known as the remodelers of the structure of the chromatin (RSC) complexes in yeast [19]. Sumoylation involves the addition of small ubiquitin-like molecules, often in chains, to the key signaling residues of the proteins. Sumoylation of the RSC forms the founder post-translational modification upon which a string of subsequent post-translational modifications may be established [20]. These are believed to control the RSC complex activity. This RSC was shown to be centrally involved in the key chromosomal functions, such as the DNA break repair, chromosomal segregation and chromosomal duplication [20]. Δ9THC inhibited this sumoylation

process directly [21], disrupting the downstream signaling through the epigenetic histone code to H3 mono-, di- and tri-methylation, H3/H4 acetylation and H2B lysine 123 ubiquitylation [20].

It was also noted that the EWAS screen showed nine hits against RAD51, which was the key member of the high-fidelity homologous recombination (HR) pathway, but only one hit against RAD52, which was part of the low-fidelity non-homologous end-joining pathway [10]. It was previously shown that the inhibition of the high-fidelity HR leads to the activation of the low-fidelity default microhomology end-joining repair pathway [22].

Thus, these many results clearly impacted and disrupted all the major functions of the chromosomes and likely provided a powerful epigenomic underpinning for the epidemiologically observed carcinogenic and teratogenic pathophysiology. Moreover, the DNA breakage was shown to be a prominent feature of the cannabis exposure of oocytes, sperm, lymphocytes and many other cells, and these finding imply that these lesions were preferentially repaired low-fidelity rather than high-fidelity pathways due to the epigenomic dysregulatory mechanisms.

3. Brain Development and Brain Aging

The Schrott EWAS study [10] revealed a widespread disruption to the receptor-based signaling, including 132 of the ionotropic AMPA receptors (GRIA), the main workhorse excitatory receptor of the CNS, 165 hits on the kainate glutamate receptor (GRIK), 26 hits on the NMDA glutamate receptor (GRIN) that mediates neuroplasticity and long-term potentiation, 11 hits on the delta glutamate receptor (GRID), 122 hits on the glutamate metabotropic receptor (GRM), 125 hits on the inhibitory GABA A receptor (GABRA), 22 hits on the GABA B receptor (GABRB), 85 hits on the “feel good” serotonin receptor (HTR), 17 hits on the dopamine receptors, five hits on each of the μ- and δ-opioid receptors and seven hits on the oxytocin “feel great” receptor.

There were ten hits each on neurexin and neuroligin, which are a ligand–receptor pair that mediate the receptor formation and scaffolding. There were eight hits on discs large homolog-associated protein 2 (DLGAP2), which is a protein known to be involved in synaptic scaffolding and the previously implicated ion autism development [23]. Similarly, there were 14 hits on the Down syndrome cell adhesion molecule (DSCAM), which is involved in axonal and dendritic pathfinding, self-avoidance and olfaction.

Recent studies have shown that the massive overgrowth of the human cerebral cortex relative to other species is controlled by signaling between the Slit-Robo ligand–receptor pair [24][25][26]. This was shown to be inhibited by cannabis [27][28][29]. There were 351 hits for the Slit signaling in the Schrott dataset and 40 hits for Robo. Moreover, there were eight hits for a key activating enzyme in this pathway—the Slit-Robo Rho GTPase activating protein (SRGAP2). These findings imply the impeded brain and neocortical outgrowth.

Another key study found that the very high gradient of retinoic acid at the frontal pole of the forebrain was responsible for driving the frontal lobe outgrowth [30]. The gradient was maintained using a retinoic acid synthesizing enzyme—alcohol dehydrogenase 1 (ALDH1) —at the frontal pole, transduced by the retinoic acid receptors RXRG and RARB, and was dissipated using the metabolic enzymes of the CYP26B1 group which had a

high concentration at the posterior of the frontal lobe and the premotor cortex. There were 13 hits in the Schrott EWAS dataset for ALDH1, ten hits for RXRG and RARB and ten hits for the CYP2 series cytochrome metabolizing enzymes.

These data showed that the cannabinoid stimulated epigenomic pathways disrupted the synaptic processing across a broad range of receptor subtypes, synaptic scaffolding using several routes, and neural progenitor and forebrain outgrowth by inhibiting several of the main pathways responsible for this key proliferation action. Such findings indicated that mental illness and congenital neurological conditions, including autistic spectrum disorders and developmental disorders such as microcephaly and anencephaly, were more likely, as observed in an increasing number of large epidemiological studies on community cannabis exposure [31][32][33][34]. Since these disorders were also characterized by impaired brain development, they may be seen as broadly degenerative in nature and, thus, consistent with an advanced broadly defined aging profile.

This was, in turn, systemically important as brain aging has been well demonstrated to drive whole organism aging [35][36][37][38]. Indeed, accelerated systemic aging accompanies many syndromes where brain aging features prominently, including progeria and Down syndrome [39][40][41][42][43].

4. Vascular Aging

The issue of vascular aging has broader implications than simply the cardiovascular system since it has been aphoristically said that “you are as old as your arteries” [35][36][44][45]. This is true not only because most people succumb to macrovascular cardiovascular disorders [46] but because most stem cell niches contain a microvascular compartment which is key to stem cell function generally.

Cannabis exposure has been shown to advance human cardiovascular age in an ecological longitudinal study [47].

The key genes in arterial development are sonic hedgehog (shh), the vascular endothelial growth factor (VEGF) and notch and ephrinB2 signaling [48].

Importantly, when investigating the EWAS-identified epigenetic methylation changes to human sperm, sonic hedgehog signaling was shown to be disrupted by nine hits on both the patched co-receptor and elsewhere, in addition to 185 hits on the key Gli3 transcription factor which signaled to the nuclear genome [10]. Notch, VEGF and ephrinB2 were disrupted at 18, five and one hits, respectively [10].

The point of these findings was not only to identify that cardiovascular development can be disrupted in these ways but that the induced arterial aging can also induce the system-wide whole organism aging processes through an impairment of the stem cell quiescence/multiplication balance both directly and indirectly.

5. Epigenomic Disruptions by Organ System

The Schrott EWAS contained 73 hits for central nervous system dysfunctions, including the brain, neurological, synaptic, cerebral, neuronal and eye derangements.

At total of 29 hits were noted for cardiovascular disorders, including the heart, atria, ventricles, atrioventricular valves and vessels.

Additionally, 22 hits were noted for orofacial genetic lesions, including the head, sensory organs, palate, nose, anterior eye and ear derangements.

Six hits were identified for limb development directly. Further exploration of a more exhaustive list of the limb morphogens revealed 130 hits for most of the key morphogens involved in limb and digit development, including the fibroblast growth factors (FGFs), retinoid signaling, Wnt signaling, bone morphogenetic pathway signaling and five genes from the sonic hedgehog (shh) pathway, namely MEGF8 (multiple EGF-like domains 8), TMEM107 (transmembrane protein 107), Gli3 (GLI gamily zinc finger 3), CHD7 (chromodomain helicase DNA-binding protein 7) and the patched receptor cofactor. Indeed, 185 hits for the key shh transcription factor Gli3 were found in the Schrott EWAS.

There were 37 hits for development of the gastrointestinal tract, including references to the esophagus, large intestine, liver and pancreas. This epigenomic pattern was noted to be consistent with the pattern of the anomalies observed in the population cannabis exposure data from both the US and Europe [31][33][49][50].

There were 23 hits observed for the urinary system, including the kidneys. When a more detailed exploration of gene regulation guided by a recent developmental renal cell map was used as a guide for data mining [51], a total of 51 hits were identified for renal development. In addition 18, 27 and 18 hits were identified for the key renal tract morphogens—notch, sonic hedgehog and transforming growth factor β, respectively.

A total of 15 hits were identified for the body wall and embryo.

Additionally, 60 hits were noted for the general otherwise unclassified disorders, including embryonic growth, DNA, mitochondria, microtubules, body trunk, body axis, ovarian reserve, breast disorders, granulocytes, myogenesis, vertebral growth and bone development.

Hence, an abundance of epigenomic evidence exists to explain the broad spectrum and high severity of the teratological patterns observed in the many jurisdictions described. These findings are described in further quantitative detail elsewhere [52].

6. Cancer Hits in the Schrott Epigenome-Wide Association Study

The Schrott supplementary file lists 487 hits for “cancer”, 112 hits for “tumor”, 126 hits for “carcinoma”, 36 hits for “neoplasm”, 32 hits for “leukemia” and 17 hits for “lymphoma”. This totals 810 hits for cancer and its synonyms,

making this one of the standout and major findings of this EWAS report.

The report specifically mentioned many leukemias, lymphomas, myeloma and tumors of the breast, ovary colorectum, thyroid, liver, brain, pancreas, melanoma, stomach, esophagus and upper aerodigestive tract.

As noted, all of these cancers have been described in association with cannabis exposure in historical [50][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69][70] and recent reports [50][53][54][55][57][58][59][71][72][73]. Further quantitative details in the description of this material is provided elsewhere [73].

By listing over 30 cancers by name and the genes whose epigenomic modulation was linked with them, these results provided a powerful pan-cancer mechanistic contributory explanation for the patterns of cancer epidemiologically observed in human populations.

It is also worth noting that many of the more recent epidemiological reports proceeded beyond the methodologies commonly adopted in observation cohort studies [50][53][54][55][57][58][59][71][72][73][74]. By applying the formal techniques of causal inference including inverse probability weighting and E-values to quantitatively exclude extraneous unmeasured confounding these investigators have constructed a pseudo-randomized analytical framework and, therefore, reported the causal relationships in preference to the more commonly noted ecological associations [50][53][54][55][57][58][59][71][72][73][74].

7. Aging Implications of the Schrott Epigenome-Wide Association Study Dataset

A concise overview and introduction to aging was provided in the preceding sections. Since DNA methylation was shown to be a key determinant of the progressive decline of the function which characterizes the aging process, and since cannabis dependence and withdrawal was shown to widely disrupt both DNA methylation and demethylation and the histone code with which it is coordinated, the disruption of the aging process itself is not unexpected. As noted above, this was confirmed in somatic tissues experimentally and found to be of high magnitude at 30% at 30 years of age [75].

It was also shown that cannabinoids can reduce the telomerase activity in a rat hepatocarcinogenesis model [76]. The Schrott EWAS dataset showed that the telomerase activity was epigenetically reduced with a significance of $p = 2.82 \times 10^{-6}$ and a multiplicity-corrected p-value of 0.01258 [10]. Indeed, since cannabis dependence inhibits TET1 ($p = 1.18 \times 10^{-5}$, multiplicity-corrected p-value = 0.02278) and this is the main counterbalancing force to the promoter hypermethylation of aging, it is easy to understand how the accelerated aging process is not only established initially, but how it might become a positive feed-forward process with time as age-related epigenomic changes are predisposed to further pro-aging epigenomic processes.

Two of the key tissues with which researchers were concerned in the present context were the male and female gametes. It was understood that none of the presently available epigenetic clocks were suitable for the application

to measure the relatively very hypomethylated ages of the gametes. However it does stand to reason that it may be possible to develop such an algorithmic clock mathematically. What the negative ages might mean, as they may relate to ages prior to birth, has yet to be determined biologically. Hence, it was not possible to measure gametal age directly or epigenomically at the time of writing.

However, it was emphasized that the well-described presence of the characteristic aging nuclear changes on the sperm of the DNA chromosomal breaks and translocations [77] and oocyte nuclear blebs and bridges [78] provided strong genetic evidence of the changes of accelerated aging. The likelihood then that both gametes and the fertilized zygote are “old prior to conception” clearly has far reaching public health and multigenerational implications in terms of the prenatal origin of many disorders in later life [79][80].

8. Strengths and Limitations

There are various strengths and limitations to the present conceptualization. The strengths include the remarkable consistency across the many epidemiological studies, which clearly demonstrates the genotoxic harms of cannabis exposure in several different international jurisdictions, in relation to both the congenital anomalies [31][33][34][49][50][81][82][83][84][85][86][87][88][89][90][91][92][93][94] and cancer [53][54][55][56][57][58][59][95], and, indeed, now also in aging [75][96]. Similar results in many different studies are clearly mutually supportive and strengthen the overall quality of the body of evidence. Similarly, there is a striking concordance between the many epigenomic studies of gestational cannabis exposure in relation to global DNA hypomethylation and the disruption of DNA methylation levels at key promoter and enhancer sites, which control the regulation of many critical genes [10][23][97][98][99][100][101][102][103][104].

The major limitation of the present work is its preliminary nature in that researchers present an introductory conceptual framework which needs to be filled out and completed by numerous further laboratory studies. The purpose of the research is merely to draw attention to this remarkable concordance of cross-disciplinary results and indicate to researchers in the basic sciences that the field is ripe for detailed exploration in many studies with far-ranging consequences.

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