EPIGENETICS
2019, VOL. 14, NO. 11, 1041–1056
https://doi.org/10.1080/15592294.2019.1633868

Taylor & Francis
Taylor & Francis Group

REVIEW

OPEN ACCESS Check for updates

Impacts of cannabinoid epigenetics on human development: reflections on Murphy et. al. 'cannabinoid exposure and altered DNA methylation in rat and human sperm' epigenetics 2018; 13: 1208-1221.

Albert Stuart Reece [a,b] and Gary Kenneth Hulse[a,b]

[a]Division of Psychiatry, University of Western Australia, Crawley, Western Australia Australia; [b]School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

ABSTRACT

Recent data from the Kollins lab ('Cannabinoid exposure and altered DNA methylation in rat and human sperm' Epigenetics 2018; 13: 1208–1221) indicated epigenetic effects of cannabis use on sperm in man parallel those in rats and showed substantial shifts in both hypo- and hyper-DNA methylation with the latter predominating. This provides one likely mechanism for the transgenerational transmission of epigenomic instability with sperm as the vector. It therefore contributes important pathophysiological insights into the probable mechanisms underlying the epidemiology of prenatal cannabis exposure potentially explaining diverse features of cannabis-related teratology including effects on the neuraxis, cardiovasculature, immune stimulation, secondary genomic instability and carcinogenesis related to both adult and pediatric cancers. The potentially inheritable and therefore multigenerational nature of these defects needs to be carefully considered in the light of recent teratological and neurobehavioural trends in diverse jurisdictions such as the USA nationally, Hawaii, Colorado, Canada, France and Australia, particularly relating to mental retardation, age-related morbidity and oncogenesis including inheritable cancerogenesis. Increasing demonstrations that the epigenome can respond directly and in real time and retain memories of environmental exposures of many kinds implies that the genome-epigenome is much more sensitive to environmental toxicants than has been generally realized. Issues of long-term multigenerational inheritance amplify these concerns. Further research particularly on the epigenomic toxicology of many cannabinoids is also required.

ARTICLE HISTORY
Received 4 May 2019
Revised 9 June 2019
Accepted 13 June 2019

KEYWORDS
Cannabinoids; cannabidiol; cannabinol; histone modifications; DNA methylation; teratogensis; autism spectrum disorder; neurobehavioural teratology; congenital cardiovascular malformations

Introduction

Physiology and pathobiology of the epigenome and its complex interactions with the genome, metabolome and immunometabolome, and cannabinoid physiopharmacology represents some of the most exciting areas of modern biological research. Type 1 and 2 cannabinoid receptors (CB1R and CB2R) are involved in a host of endogenous processes with potential therapeutic applications in numerous fields as diverse as pain, nausea, temperature regulation and weight control amongst others. Several recent detailed structural descriptions of the CB1R and CB2R complexed with high affinity agonists and antagonists [1,2], and pathways for the bulk biological synthesis of cannabinoids [3] open the way to the rational design of high affinity molecules to differentially modulate these key receptors which are involved in a host of endogenous processes with diverse potential therapeutic applications. The use of exogenous cannabinoid compounds that bind to CB1R and CB2R may however also produce unwanted side effects including through modulation of DNA methylation states.

Within each nucleated cell, 2 m of DNA is normally stored coiled around four histones known as a nucleosome. A total of 147 bases of DNA are wrapped twice around two sets of H2A, H2B, H3 and H4 which together form the histone octamer. The bases of DNA itself may have a methyl group (CH_3-) attached to them, usually to cytosine-phosphate-guanine (CpG), which when it occurs in the region of the gene promoter, blocks the transcription machinery and prevents the gene from becoming activated. The tails of the four histone proteins protrude from the central globular core and normally bind by electrostatic forces to the coiled DNA. Addition of an acetyl group to these

CONTACT Albert Stuart Reece stuart.reece@bigpond.com 39 Gladstone Rd., Highgate Hill, Brisbane, Queensland, Australia

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

histone tails, particularly on H3 and H4, disrupts the salt bridges opening up the DNA code for active transcription. Histone tails can also be methylated or indeed be modified by many groups (mono-, di- and trimethyl, acetyl, phosphoryl, crotonyl, citrulline, ubiquitin and ADP-ribosyl, etc.) which control gene transcription [4]. DNA is transcribed into RNA some of which is made into the many proteins from which our bodies are made. However, much of the RNA also has purely informatic roles, and short and long non-coding RNA's (ncRNA) controls DNA availability and transcription, RNA processing and splicing and can form a scaffold upon which layers of DNA regulation can be built [5,6]. These various mechanisms, DNA methylation, post-translational modification of histone tails, nucleosome positioning, histone replacement, nuclear positioning and ncRNA's form the basis of epigenetic regulation [7,8] and appear to undergo an 'epigenetic conversation' amongst these different layers [4]. Chromatin loops are extruded through cohesin rings giving rise to transcription factories (topologically active domains) where different regions of the DNA including proximal promoters and distal enhancers are brought into close proximity to control transcription either on the same chromosome (in *cis*) or sometimes on nearby chromosomes (in *trans*). Super-enhancers, enhancer cross-talk, and extensive 3D remodelling of euchromatin looping during development are also described [9–14].

Transgenerational inheritance

Moreover, a variety of studies in animals and several epidemiological studies in humans show that the epigenetic code can form a mechanism for inheritable changes across generations from both father and mother to subsequent generations which do not involve changes in the genetic code itself. Such epigenetic inheritance has been shown clinically for starvation, obesity, bariatric surgery and for tobacco and alcohol consumption [7,15–17]. It has also been demonstrated in rodents for alcohol, cocaine and opioids, and in rodents' immune system, nucleus accumbens and sperm following cannabinoid exposure in the parents [18–23].

If DNA is thought of as the cells' bioinformatic 'hardware' then the epigenome can be considered its programming 'software'. The epigenome controls gene expression and is key to cell differentiation into different tissue fates [24], different states of cellular differentiation, to cellular reprogramming into induced pluripotential stem cell states [25–29], cancer [30–32], numerous neuropsychiatric diseases including addiction [4,33–35], immune, metabolic and brain memory [4,36–39], aging [40], and the response of the cell to changes in its environment by way of gene-environment interactions [7,16,19] including the development of so-called 'epigenetic scars' [4].

Direct epigenomic sensing of the environment

This powerful informatic system has recently been shown to have a host of unforeseen capabilities. It has been shown that histone tails sense oxygen tension rapidly within 1 h with resulting modification of gene expression cassettes [41]. Lysine (K) demethylase 5A (KDM5A) is a Jumanji-C domain containing molecular dioxygenase which is inactivated by hypoxia in a hypoxia-inducible factor-independent manner, controls H3K4me3 and H3K36me3 histone trimethylations and governs the transcriptome expression several hours after brief hypoxia. Similarly, KDM6A is also an oxygen sensitive dioxygenase and histone demethylase which controls H3K27me3. Its blockade by hypoxia interferes with cell differentiation and maintains cells in an undifferentiated state [42]. Since the ten eleven translocase enzymes and are key demethylators of DNA and are dioxygenases also sensitive to profound hypoxia, and since hypoxia exists in most stem cell niches and at the centre of many tumours, such histone- and DNA-centred mechanisms are likely to be important in stem cell, aging, cellular differentiation and cancer biology.

Epigenomic regulation of tumour immunometabolome

Similarly, one of the great paradoxes of cancer biology is the presence within tumours of numerous effector T-cells which are able to expand and eradicate large metastatic tumours effectively, but do not do so within clinical cancers. It was recently shown that this effect is due to the very elevated nucleocytosolic potassium level within tumour

lymphocytes which stalls metabolism and runs down acetyl-coenzyme A levels, the main acetyl donor for histone acetylation and induces a form of calorie restriction (like starvation) including autophagy and mitophagy and impairs the normal mTOR (mammalian target of rapamycin)-dependent T-cell receptor-mediated activation response [43]. This program was mediated by reduced levels of H3K9 and H3K27 acetylation. Hence, tumour lymphocyte anergy and stemness were both mediated epigenetically and were shown to be reversible when the immunometabolic defect was corrected either genetically or by substrate supplementation. This work elegantly demonstrates the close relationship between the metabolic state of cells, cell differentiation state and starvation response, the control of cell fate by the epigenetic landscape and disease outcome.

Metabolomic supply of epigenetic substrate

Several studies similarly link the supply of metabolic intermediates required as inputs by the epigenetic machinery to epigenetic state and downstream gene control. Indeed, the well-known supplementation of staple foods by folic acid is believed to act because of the central role played by this vitamin in the methyl cycle and the supply of single carbon units to the methylation machinery for DNA and histones. A moments reflection shows that expression of the DNA of the mitochondria and the DNA of the nucleus need to be tightly coordinated to supply the correct number of subunits for the complex machineries of the mitochondrion including electron transport. This mitonuclear balance acts at several levels including RNA transfer, metabolic substrate (acetyl-coenzyme A, nicotinamide mononucleotide) transfer and the control of the epigenetic regulators PARP (polyadenosineribosyl polymerase) and Sirt1 (a major histone deacetylase) [44].

Cannabinoid signalling impacts mitochondria

As noted above the identification of CB1R and CB2R on the plasma membrane has been a major milestone in cellular cannabinoid physiology. It is less well known that CB1R's also exist on the mitochondrial outer membrane, and that the inner and outer leaflet of the mitochondria, together with the intermembrane space host the same cannabinoid transduction machinery as the plasmalemma [45–47]. Neuronal mitochondrial CB1R's have been implicated in memory and several critical neural processes [48–50]. Hence, the well-substantiated findings that diverse cannabinoids generally suppress mitochondrial activity (in neurons, lung, liver and sperm), lower the mitochondrial transmembrane potential and interfere with oxidative phosphorylation [51–53] carry major epigenetic implications not only for mitonuclear balance and trafficking including the mitochondrial stress response, but also for the supply of the requisite metabolic intermediates in terms of acetyl-coenzyme A which is an absolute requirement for histone acetylation and normal gene activation.

Histone serotonylation and dopaminylation

Serotonin, which has long been implicated in mood dysregulation and drug addiction was recently shown to act as a novel post-translational modification of the tail of H3 at lysine 4 via serotonylation where it increases the binding of the transcription machinery and allows correct cell differentiation [54,55]. It is likely that dopamine will soon be similarly implicated [54,55].

Epigenomics in cancer

Almost accompanying the modern bioinformatic explosion of knowledge related to the sequencing of the human genome has been a parallel increase in knowledge of the complexities and intricacies of epigenomic regulation. Nowhere is this more evident than in cancer. Indeed, it has become apparent that there are numerous forms of cross-talk, interaction and cross-regulation between the genome and the epigenome and the two are in fact highly interrelated. This is of particular relevance to chromosomal integrity and cancerogenic mechanisms. Several mechanisms have been described for such interactions including alterations of DNA methylation, altered cytosine hydroxymethylation [56], alteration of TERT function which is a key catalytic component of the telomerase enzyme which protects chromosome ends [57] and altered architecture of enhancers

and their looping interactions with promoters which control gene expression [12,58,59]. Indeed, pharmacological modulation of the bromodomain 'readers' of epigenomic information has become a very exciting area within modern cancer therapeutic research [12,59–68], and forms an area into which large pharmaceutical companies are presently investing several billion dollars [69,70].

Gamete cannabinoid epigenomics – Murphy et. al

In this powerful context, the masterful epigenetic work from the Kollins laboratory of Murphy and colleagues was situated [71]. These workers studied 12 control men who self-reported no psychoactive drug use in the last 6 months, and 12 subjects who reported more than weekly use of cannabis only, with all results confirmed by urine toxicology and ultra performance liquid chromatography/tandem mass spectrometry and enzyme immunoassay. In parallel two groups of 9-week-old male rats were administered solvent or 2 mg/kg THC by gastric lavage for 12 days prior to sacrifice and the epididymis was harvested. Sperm were assayed by the 'swim out' method where sperm swam out into normal saline bath solution. Cannabis exposed men had lower sperm counts, and it was found that there was differential sperm DNA methylation at 6,640 CpG sites including at 3,979 CpG islands in gene promoters where methylation was changed by more than 10% (which is alot). Significant changes were in both the hypomethylation and hypermethylation direction were noted with the changes in the hypomethylation group being more marked across the genome and at gene promoters. Pathways in cancer (including the *BRAF, PRCACA, APC2 PIK3R2, LAMA1, LAMB1, AKT1* and *FGF* genes), hippo pathways (which are also important in cancer and in embryonic body pattern formation), the MAP kinase pathway (also involved in growth and cancer), AMPA, NMDA and kainate glutamate receptor subunits, and the *Wnt genes 3A, 5A, 9A, 10A* (involved in cancer and in body patterning and morphogensis) were found to be particularly affected. A dose–response effect was demonstrated at 183 CpG sites on 177 genes including the *PTG1R* gene which encodes the prostacyclin (a powerful vasodilator and antithrombotic agent) receptor which was down-regulated. Twenty-three genes involved in platelet activation and 21 genes involved in glutamate metabolism were also modulated. *LAMB1*, whose gene product laminin B has been implicated in progeria and is increasingly implicated in genetic ageing pathways through its role in nuclear positioning of chromatin and the maintenance of heterochromatin (including female X-chromosome inactivation) in an inactive state inside the nuclear membrane, and its role in establishing integrity of the nuclear envelope, was also identified [72]. Results in the rats closely paralleled those found in humans. Fifty-five genes were found to overlap between altered sperm methylation patterns and a previous study of brain Nuclear Accumbens DNA methylation in prenatally cannaboid exposed rats which showing increased heroin self-administration, a highly statistically significant result. These results support the hypothesis that the transgenerational transmission of defects following pre-conceptual exposure to cannabis found in the immune system and limbic system of the brain including increased tendency for drug use in later life in rodents [18,19] may be transmitted through alterations in the DNA methylation of the male germ line. More work is clearly needed in this area with exhaustive epigenetic, transcriptomic and genomic characterization of these results with larger sample sizes and in other species.

Cannabis – cancer links

Mechanistically these results have very far-reaching implications indeed and appear to account for much of the epidemiologically documented associations of cannabis use. Cannabis has been associated with cancer of the mouth and throat, lung, bladder, leukaemia, larynx, prostate and cervix [73] and in four out of four studies with testicular teratomas [74–77] with a relative risk of three in meta-analysis [78]. Cannabis has also been implicated with increased rates of the childhood cancers acute lymphocytic leukaemia, acute myeloid leukaemia, acute myelomonocytic leukaemia, neuroblastoma and rhabdomyosarcoma [73].

These are believed to be due to inheritable genetic or epigenetic problems from the parents [79,80], albeit the mechanism of such transmission was not understood in the pre-epigenomic era.

Results of Murphy and colleagues [71] may potentially explain mechanistically much of the epidemiologically documented morbidity that has in the past been associated with cannabis use. As noted, cannabis contains the same tars as tobacco and also several known genotoxic compounds, and is also immunoactive. Such actions imply several mechanisms by which cannabis may be implicated in carcinogenic mechanisms.

That cannabis is associated with heritable paediatric cancers where the parents themselves do not harbour such tumours is suggestive evidence that non-genetic and likely epigenetic mechanisms are involved in the childhood cancers which are observed. Detailed delineation of such putative pathways will require further research.

Cannabis has also been shown to be associated with increased rates of gastroschisis in seven of seven studies to examine this association [81–87]. This pathology, where the bowels of the neonate protrude through the abdominal wall usually to the right of the umbilicus, is believed to be due to a disruption of blood flow to the forming abdominal wall. If cannabinoid exposure powerfully activates platelets through multiple mechanisms and disrupts major vasodilator systems such as the prostacyclin receptor then such a pathway could well damage the tiny blood vessels of the developing foetus and account for the development of gastroschisis. Cannabis use in adults has been linked with both myocardial infarction and stroke possibly by similar mechanisms [48,88]. It has been shown elsewhere that cannabis use can also stimulate inflammation and be proinflammatory [89].

Epigenomics of foetal alcohol syndrome

Indeed, foetal alcohol syndrome disorder (FASD) is said to be mediated in part by the CB1R [90–92], to be epigenetically mediated [93–96], and to comprise amongst other features small heads, microcephaly, impaired visuospatial coordination and to be commonly associated with ventricular septal defect and atrial septal defect [97] all of which have been described in association with prenatal cannabis exposure [83,98–101]. However, the facial features of FASD are not described in the congenital cannabis literature.

Cannabis and congenital anomalies

Indeed, one Hawaiian statewide epidemiological report found elevated rates of 21 congenital defects in prenatally cannabis exposed infants [83]. Whilst this paper is unique in the literature it helps explain much about the presently reported patterns of congenital anomalies across USA in relation to atrial septal defect, Downs' syndrome, Trisomy 18, ventricular septal defect, limb reduction defects, anotia, gastroschisis [102] and autism [103], all of which crude rates are more common in states with liberal cannabis policies. Similar morbidity patterns were observed in Canada with crude rates of all congenital defects, gastroschisis, total cardiovascular defects and orofacial clefts [104] more common in areas with higher cannabis use [105]. The Colorado birth defects registry has also reported a three-fold increase in the crude (unadjusted) rate of atrial septal defects 2000–2014 spanning the period of cannabis legalization together with increases of 30% or more over the same period in crude rates of total cardiovascular defects, ventricular septal defects, Down's syndrome and anencephaly [106]. This is highly significant as atrial septal defect has only been found to be linked with cannabis in the Hawaiian study, suggesting that our list of cannabis-related defects is as yet incomplete. As mentioned above the putative link between atrial septal defect and cannabis use has also been found in the generality of states across the USA [102]. It should also be noted that according to a major nationally representative recurrent survey the use of all other drugs in Colorado fell during this period, making cannabis the most likely pharmacological suspect for the surge in congenital anomalies [107–109].

These findings are also consistent with data arising from France, wherein three separate regions which have permitted cannabis to be used as feed for the dairy industry calves are born without legs, and an increase in the rate of phocomelia (no arms) in human infants has similarly been observed. In the French northeast region of Ain which is adjacent to Switzerland, the crude rate of phocomelia is said to be elevated 58 times above background [110,111], whilst in nearby Switzerland which has not permitted cannabis to be used as a feed crop no such anomalies are observed.

Neuroteratogenesis and beyond

The above comments in relation to epigenetic modulation of the glutamate system have been shown in recent studies to be related to many neuropsychiatric disorders. However, the recent demonstration at least in insects that glutamate could also act as a key morphogen in body patterning processes and major organ formation may have much wider implications well beyond the neuraxis [112].

Cannabis and epigenetic ageing

The finding of overall DNA hypomethylation by Murphy's group [71] carries particular significance especially in the context of disordered lamin B metabolism. Chronic inflammation is known to be a major risk factor for carcinogenesis in humans in many organs including the skin, oropharynx, bronchi, lungs, oesophagus, stomach, pancreas, liver, biliary tree, colon, bladder and prostate [113–116]. Inflammatory conditions are invariably strongly pro-oxidative and damage to DNA is not unusual. Because CpGs in gene promoters are more often largely unmethylated and therefore exposed the guanine in these positions is a common target for oxidative damage. Oxo-guanine is strongly mutagenic. This form of DNA damage recruits the maintenance DNA methyltransferase DNMT1 from the gene body to the gene promoter. There DNMT1 recruits Sirt1, a histone deacetylase which tends to epigenetically silence gene expression, and also EZH2 part of the polycomb repressive complexes 2 and 4 which epigenetically silences gene expression and tends to spread the silencing of chromatin. Hence, one of the end results of this form of oxidative DNA damage is to move the DNA methylation from the gene bodies to the gene promoters, thereby hypermethylating the promoters [117], the CpG Island Methylator Phenotype (CIMP) and hypomethylating the gene bodies and intergenic regions [118]. By this epigenetic means chronic inflammation and tobacco smoke have been shown to induce widespread epigenomic field change right across tissues such as colon, bronchi or bone marrow [116,119,120]. Furthermore, this mechanism moves gene expression from the control of histone modification to DNA methylation which tends to be more fixed and less plastic than histone alterations. Such findings are consistent with a previous demonstration of accelerated ageing in cannabis exposed clinical populations [121].

Epigenomic control of mobile transposable genetic elements

Reducing the global level of DNA methylation also has the effect of reducing the control of mobile transposable repeat elements in the genome [122]. Forty-two per cent of the human genome has been shown to be comprised of these mobile elements of various varieties. Long Interspersed Repeat Elements (LINE-1) are believed to be retroviral repeat elements which long ago became incorporated in the genome and are able when expressed to induce their own reverse transcription back into the genome via endogenous reverse transcriptases [122]. For this reason, they are also called 'jumping genes.' Because they become randomly incorporated into the genome after reverse transcription their activity is very damaging to genetic integrity. Whilst retrotransposon mobility is normally controlled by three mechanisms these defences can be overcome in advanced cellular senescence. The presence of double-stranded DNA (dsDNA) in the cytoplasm is strongly stimulating for the immune system and stimulates a type-1 interferon proinflammatory response, which further exacerbates the cycle and directly drives the Senescence Associated Secretory Phenotype (SASP) of advanced senescence and the 'inflamm-aging' which is well described in advanced age [123–125]. Accelerated ageing in patients exposed clinically to cannabis has previously been described using a well validated metric of arterial stiffness [126]. Whilst neither Murphy [71] nor Watson [20] found evidence following cannabinoid exposure for altered methylation of repeat elements the presence of chronic inflammation in the context of widespread preneoplastic change and documented neoplasia suggest that this newly described ageing mechanism might well merit further investigation.

These changes are likely exacerbated by several classical descriptions that cannabinoids reduce the overall level of histone protein synthesis [127–129]. Since the overall length of DNA does not change this is likely to further open up the genome

to dysregulated transcription. Severe morphological abnormalities of human and rodent sperm have been reported [127,130–132].

Cannabinoids and oocytes

Similarly classical descriptions exist of grossly disrupted mitoses, particularly in oocytes [133], which are said to be seriously deficient in DNA repair machinery [134–136]. Morishima reported as long ago as 1984, evidence of nuclear blebs and bridges due to deranged meiotic divisions in cannabinoid-exposed rodent oocytes [133]. Similar blebs and bridges have been reported by others [128,129,137]. It has since been shown that these nuclear blebs represent areas of weakness of the nuclear membrane which are often disrupted spilling their contents into the cytoplasm [72]. They are also a sign of nuclear ageing.

Cannabinoids and micronuclei

Cannabis has long been known to test positive in the micronuclear assay due to interference with the function of the mitotic spindle [138–140]. This is a major cause of chromosomal disruption and downstream severe genetic damage in surviving cells [141,142], has previously been linked with teratogenesis and carcinogenesis, and which is also potently proinflammatory by releasing dsDNA into the cytoplasm and stimulating cGAS-STING (Cyclic GMP-AMP synthase – STimulator of INterferon Gamma) signalling and downstream innate immune pathways [143–146]. Cytoplasmic dsDNA has also been shown to be an important factor driving the lethal process of cancer metastasis [147].

Cannabis and wnt signalling

The findings of Murphy in relation to Wnt signalling are also of great interest [71]. It has been found by several investigators that prenatal cannabis exposure is related to encephalocoele or anencephaly [83,148,149]. Non-canonical Wnt signalling has been shown to control the closure of the anterior neuropore [150] providing a mechanistic underpinning for this fascinating finding. Wnt signalling has also been implicated in cancer development in numerous studies [151–154] and in controlling limb development [155] which have been previously linked with cannabis exposure (as noted above).

Cannabis and autism

It was recently demonstrated that the rising use of cannabis parallels the rising incidence of autism in 50 of 51 US states and territories including Washington D.C., and that cannabis legalization was associated with increased rates of autism in legal states [108,109]. Several cannabinoids in addition to Δ9-tetrahydrocannabinol (THC) were implicated in such actions including cannabidiol, cannabinol, cannabichromene, cannabigerol and tetrahydrocannabivarin. A rich literature demonstrates the impacts of epigenomics on brain development and its involvement in autistic spectrum disorders [156–161]. Whether cannabis is acting by epigenetic or other routes including those outlined above remains to be demonstrated. Further research is indicated.

Cannabidiol and other cannabinoids

These findings raise the larger issue of the extent to which the described changes reflect the involvement of THC as compared to other cannabinoids in the more general genotoxicity and epigenotoxicity of both oral (edible) and inhaled (smoked) cannabis. THC, cannabidiol, cannabidivarin, and cannabinol have previously been shown to be genotoxic to chromosomes and associated with micronucleus development [162,163]. American cannabis has been selectively bred for its THC content and the ratio of THC to cannabidiol (CBD) was noted to have increased from 14:1 to 80:1 1998–2018 [71]. However in more recent times, cannabidiol is being widely used across the USA for numerous (nonmedical) recommendations.

Cannabidiol is known to inhibit mitochondrial oxidative phosphorylation including calcium metabolism [47,164–171] which is known to have a negative effect on genome maintenance and is believed to secondarily restrict the supply of acetyl and other groups for epigenetic modifications. Cannabidiol is known to act via CB1R's particularly at higher doses [166,172–179]. Cannabidiol acts via PPARγ (Peroxisome Proliferator Activator

Receptor) [180–187] which is a nuclear receptor which is implicated in various physiological and pathological states including adipogenesis, obesity, diabetes, atherogenesis, neurodegenerative disease, fertility and cancer [188]. In a human skin cell culture experiment, cannabidiol was shown to act via CB1R's as a transcriptional repressor by increasing the level of global DNA methylation by enhancing the expression of the maintenance DNA methylase DNMT1 which in turn suppressed the expression of skin differentiation genes and returned the cells to a less differentiated state [179]. One notes, importantly, that this DNA hypermethylation paralleled exactly the changes reported by Murphy for THC hypermethylation [71]. The de-differentiation reported or implied in both studies is clearly a more proliferative and proto-oncogenic state. Hence, while more research is clearly required to carefully delineate the epigenetic actions of cannabidiol, its activity at CB1R's, its mitochondrial inhibitory action, its implication of PPARγ and particularly its THC-like induction of epigenetic and cellular de-differentiation, together with its implication in chromosomal fragmentation and micronucleus induction would suggest that caution is prudent whilst the results of further research are awaited.

Other cannabinoid receptors and notch signalling

The above discussion is intended to be indicative and suggestive rather than exhaustive as the cannabinoids' pharmacological effects are very pleiotropic, partly because CB1R's, CB2R's – and six other cannabinoid sensing receptors [189]– are widely distributed across most tissues. One notes that the mechanisms described above do not obviously account for very important finding that in both Colorado [106,107] and Canada [190–192] increasing rates of cannabis use were associated with higher rates of total congenital cardiovascular disease. One observes that in both cases the cited rise in rates refers to an elevation of crude rates unadjusted for other covariates. This finding is important for several reasons not the least of which is that cardiovascular disease is the commonest class of congenital disorders. It may be that this action is related to the effects of cannabinoids binding high-density endovascular CB1R's from early in foetal life [193] and interacting with the notch signalling system [194–196]. Notch is a key morphogen involved in the patterning particularly of the brain, heart, vasculature and haemopoietic systems [197] and also in many cancers. Notch signalling both acts upon the epigenome and is acted upon by the epigenome both in benign (atherosclerotic and haemopoietic) [198,199] and cancerous (ovarian, biliary, colonic, leukaemic) diseases [200–204]. Clearly in view of their salience, the interactions between cannabinoids and both notch and Wnt signalling pathways constitute fertile areas for ongoing research.

Conclusion

In short the timely paper by Murphy and colleagues [71] nicely fills the gap between extant studies documenting that pre-conception exposure to cannabis is related to widespread changes in epigenetic regulation of the immune and central nervous systems and confirms that male germ cells are a key vector of this inheritance and has given new gravity to epidemiological data on the downstream teratological manifestations of prenatal cannabinoid exposure. The reasonably close parallels in findings between rats and man confirm the usefulness of this experimental model. Since guinea pigs and white rabbits are known to form the most predictive preclinical models for human teratogenicity studies [205,206] it would be prudent to investigate how epigenomic results in these species compared to those identified in man and rodents. Finally the considerable and significant clinical teratogenicity of cannabis, including its very substantial neurobehavioural teratogenicity imply that such studies need to be prioritized by the research community and the research resourcing community alike, particularly if the alarming findings of recent European experience in terms of cannabinoids allowed in the food chain is not to be repeated elsewhere. Indeed, the recent passage of the nearly $USD1trillion USA Farm Act which encourages hemp to be widely grown for general use together with the advent in some US cafés of 'hempburgers' and 'cannabis cookies' would appear to have ushered in just such an era. Hemp oil has recently been marketed in

Australian supermarkets completely unsupervised. Meanwhile, the rapidly accumulating and stellar discoveries relating to the pathobiology of the epigenome and its remarkable bioinformatical secrets continue to be of general medical and community importance. In some areas, particularly relating to the epigenotoxicology of the non-THC cannabinoids, further research is clearly indicated, especially in view of the widespread use and relatively innocuous reputation of cannabis derivates including particularly cannabidiol.

Such issues suggest that in the pharmacologically exciting era of the development of novel intelligently designed cannabinoids intended for human therapeutics, considerations of genomic and epigenomic toxicity including mutagenicity, teratogenicity, carcinogenicity, pro-ageing and heritable multigenerational effects warrant special caution and attention prior to the widespread exposure of whole populations either to phytocannabinoids or to their synthetic derivatives. Equally, the possibility of locus-specific epigenetic medication development as modifiers of the epigenetic reading, writing and erasing machinery suggests that very exciting developments are also beginning in this area [4].

Author Note

While this paper was in review our paper examining the epidemiological pattern and trends of Colorado birth defects of 2000-2014 and entitled "Cannabis Teratology Explains Current Patterns of Coloradan Congenital Defects: The Contribution of Increased Cannabinoid Exposure to Rising Teratological Trends" was accepted by the journal Clinical Pediatrics. It provides further details and confirmation on some of the issues discussed in the present paper. It also contains a detailed ecological investigation of the role of cannabidiol at the epidemiological level which confirms and extends the mechanistic observations and the quantitative remarks relating to the epidemiology of birth defects in Colorado made in the present manuscript. The interested reader may also wish to consult this resource.

Disclosure statement

No potential conflict of interest was reported by the authors.

Financial Disclosure

The authors have indicated they have no financial relationships to this article to disclose.

ORCID

Albert Stuart Reece http://orcid.org/0000-0002-3256-720X

References

[1] Krishna Kumar K, Shalev-Benami M, Robertson MJ, et al. Structure of a signaling cannabinoid receptor 1-G protein complex. Cell. 2019 Jan 24;176(3):448–458 e12.
[2] Li X, Hua T, Vemuri K, et al. Crystal Structure of the Human Cannabinoid Receptor CB2. Cell. 2019 Jan 24;176(3):459–467 e13.
[3] Luo X, Reiter MA, d'Espaux L, et al. Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. Nature. 2019 Mar;567(7746):123–126.
[4] Nestler EJ, Luscher C. The Molecular Basis of Drug Addiction: linking Epigenetic to Synaptic and Circuit Mechanisms. Neuron. 2019 Apr 3;102(1):48–59.
[5] Rinn JL, Kertesz M, Wang JK, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007 Jun 29;129(7):1311–1323.
[6] Kotake Y, Nakagawa T, Kitagawa K, et al. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. Oncogene. 2011 Apr 21;30(16):1956–1962.
[7] Szutorisz H, Hurd YL. High times for cannabis: epigenetic imprint and its legacy on brain and behavior. Neurosci Biobehav Rev. 2018 Feb;85:93–101.
[8] Ohno M, Ando T, Priest DG, et al. Sub-nucleosomal Genome Structure Reveals Distinct Nucleosome Folding Motifs. Cell. 2019 Jan 24;176(3):520–534 e25.
[9] Mateo LJ, Murphy SE, Hafner A, et al. Visualizing DNA folding and RNA in embryos at single-cell resolution. Nature. 2019 Apr;568(7750):49–54.
[10] Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013 Apr 11;153(2):320–334.
[11] Pefanis E, Wang J, Rothschild G, et al. RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. Cell. 2015 May 7;161(4):774–789.
[12] CJ O, Federation AJ, Schwartz LS, et al. Enhancer Architecture and Essential Core Regulatory Circuitry of Chronic Lymphocytic Leukemia. Cancer Cell. 2018 Dec 10;34(6):982–995 e7.
[13] Fulciniti M, Lin CY, Samur MK, et al. Non-overlapping Control of Transcriptome by Promoter-

and Super-Enhancer-Associated Dependencies in Multiple Myeloma. Cell Rep. 2018 Dec 26;25 (13):3693–3705 e6.
[14] Mack SC, Pajtler KW, Chavez L, et al. Therapeutic targeting of ependymoma as informed by oncogenic enhancer profiling. Nature. 2018 Jan 4;553 (7686):101–105.
[15] Szutorisz H, Egervari G, Sperry J, et al. Cross-generational THC exposure alters the developmental sensitivity of ventral and dorsal striatal gene expression in male and female offspring. Neurotoxicol Teratol. 2016;58:107–114.
[16] Szutorisz H, Hurd YL. Epigenetic Effects of Cannabis Exposure. Biol Psychiatry. 2016 Apr 1;79(7):586–594.
[17] Cecil CA, Walton E, Smith RG, et al. DNA methylation and substance-use risk: a prospective, genome-wide study spanning gestation to adolescence. Transl Psychiatry. 2016 Dec 6;6(12):e976.
[18] DiNieri JA, Wang X, Szutorisz H, et al. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biol Psychiatry. 2011 Oct 15;70(8):763–769.
[19] Szutorisz H, DiNieri JA, Sweet E, et al. Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. Neuropsychopharmacology. 2014 May;39 (6):1315–1323.
[20] Watson CT, Szutorisz H, Garg P, et al. Neuropsychopharmacology. In: Genome-Wide DNA Methylation Profiling Reveals Epigenetic Changes in the Rat Nucleus Accumbens Associated With Cross-Generational Effects of Adolescent THC Exposure. Jun, 2015;40(13):2993–3005.
[21] Yang X, Hegde VL, Rao R, et al. Histone modifications are associated with Delta9-tetrahydrocannabinol-mediated alterations in antigen-specific T cell responses. J Biol Chem. 2014 Jul 4;289 (27):18707–18718.
[22] Govorko D, Bekdash RA, Zhang C, et al. Male germline transmits fetal alcohol adverse effect on hypothalamic proopiomelanocortin gene across generations. Biol Psychiatry. 2012 Sep 1;72(5):378–388.
[23] Fullston T, Ohlsson Teague EM, Palmer NO, et al. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. Faseb J. 2013 Oct;27(10):4226–4243.
[24] Waddington CH. The Epigenotype. Endeavour. 2019;1942:18–20.
[25] Beerman I, Rossi DJ. Epigenetic Control of Stem Cell Potential during Homeostasis, Aging, and Disease. Cell Stem Cell. 2015 Jun 4;16(6):613–625.
[26] Brumbaugh J, Hochedlinger K. Removing reprogramming roadblocks: mbd3 depletion allows deterministic iPSC generation. Cell Stem Cell. 2013 Oct 3;13 (4):379–381.
[27] Ding J, Huang X, Shao N, et al. Tex10 Coordinates Epigenetic Control of Super-Enhancer Activity in Pluripotency and Reprogramming. Cell Stem Cell. 2015 Jun 4;16(6):653–668.
[28] Mansour AA, Gafni O, Weinberger L, et al. The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. Nature. 2012 Aug 16;488(7411):409–413.
[29] Snoeck HW. Can Metabolic Mechanisms of Stem Cell Maintenance Explain Aging and the Immortal Germline?. Cell Stem Cell. 2015 Jun 4;16(6):582–584.
[30] Huang SS, Clarke DC, Gosline SJ, et al. Linking proteomic and transcriptional data through the interactome and epigenome reveals a map of oncogene-induced signaling. PLoS Comput Biol. 2013;9(2):e1002887.
[31] Ziech D, Franco R, Pappa A, et al. Reactive oxygen species (ROS)–induced genetic and epigenetic alterations in human carcinogenesis. Mutat Res. 2011 Jun 3;711(1–2):167–173.
[32] Lao VV, Grady WM. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol. 2011 Dec;8 (12):686–700.
[33] Dong X, Liao Z, Gritsch D, et al. Enhancers active in dopamine neurons are a primary link between genetic variation and neuropsychiatric disease. Nat Neurosci. 2018 Oct;21(10):1482–1492.
[34] Guennewig B, Bitar M, Obiorah I, et al. THC exposure of human iPSC neurons impacts genes associated with neuropsychiatric disorders. Transl Psychiatry. 2018 Apr 25;8(1):89.
[35] Kozlenkov A, Jaffe AE, Timashpolsky A, et al. DNA Methylation Profiling of Human Prefrontal Cortex Neurons in Heroin Users Shows Significant Difference between Genomic Contexts of Hyper- and Hypomethylation and a Younger Epigenetic Age. In: Genes (Basel), Vol. 8. 2017 May 30. p. 6.
[36] Heyward FD, Gilliam D, Coleman MA, et al. Obesity Weighs down Memory through a Mechanism Involving the Neuroepigenetic Dysregulation of Sirt1. J Neurosci. 2016 Jan 27;36(4):1324–1335.
[37] Villeneuve LM, Reddy MA, Lanting LL, et al. Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. Proc Natl Acad Sci U S A. 2008 Jul 1;105(26):9047–9052.
[38] Halder R, Hennion M, Vidal RO, et al. DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. Nat Neurosci. 2016 Jan;19 (1):102–110.
[39] Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003 Mar;33:Suppl:245–54.
[40] Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.
[41] Batie M, Frost J, Frost M, et al. Hypoxia induces rapid changes to histone methylation and reprograms chromatin. Science. 2019 Mar 15;363(6432):1222–1226.

[42] Chakraborty AA, Laukka T, Myllykoski M, et al. Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate. Science. 2019 Mar 15;363(6432):1217–1222.
[43] Vodnala SK, Eil R, Kishton RJ, et al. T cell stemness and dysfunction in tumors are triggered by a common mechanism. Science. 2019;363(6434):eaau0135.
[44] Canto C, Menzies KJ, Auwerx J. NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. Cell Metab. 2015 Jul 7;22(1):31–53.
[45] Benard G, Massa F, Puente N, et al. Mitochondrial CB (1) receptors regulate neuronal energy metabolism. Nat Neurosci. 2012 Mar 4;15(4):558–564.
[46] Hebert-Chatelain E, Desprez T, Serrat R, et al. A cannabinoid link between mitochondria and memory. Nature. 2016 Nov 24;539(7630):555–559.
[47] Hebert-Chatelain E, Reguero L, Puente N, et al. Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB1 receptor. Mol Metab. 2014 Jul;3(4):495–504.
[48] Wolff V, Schlagowski AI, Rouyer O, et al. Tetrahydrocannabinol induces brain mitochondrial respiratory chain dysfunction and increases oxidative stress: a potential mechanism involved in cannabis-related stroke. Biomed Res Int. 2015;2015:323706.
[49] Djeungoue-Petga MA, Linking Mitochondria H-CE. Synaptic Transmission: the CB1 Receptor. Bioessays. 2017 Dec;39(12). doi: 10.1002/bies.201700126. Epub 2017 Oct 23.
[50] Ruggiero A, Aloni E, Korkotian E, et al. Loss of forebrain MTCH2 decreases mitochondria motility and calcium handling and impairs hippocampal-dependent cognitive functions. Sci Rep. 2017 Mar 9;7:44401.
[51] Sarafian TA, Kouyoumjian S, Khoshaghideh F, et al. Delta 9-tetrahydrocannabinol disrupts mitochondrial function and cell energetics. Am J Physiol. 2003 Feb;284(2):L298–306.
[52] Sarafian TA, Habib N, Oldham M, et al. Inhaled marijuana smoke disrupts mitochondrial energetics in pulmonary epithelial cells in vivo. Am J Physiol. 2006 Jun;290(6):L1202–9.
[53] Rossato M, Ion Popa F, Ferigo M, et al. Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. J Clin Endocrinol Metab. 2005 Feb;90(2):984–991.
[54] Cervantes M, Sassone-Corsi P. Modification of histone proteins by serotonin in the nucleus. Nature. 2019 Mar;567(7749):464–465.
[55] Farrelly LA, Thompson RE, Zhao S, et al. Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. Nature. 2019 Mar;567(7749):535–539.
[56] Bhattacharyya S, Pradhan K, Campbell N, et al. Altered hydroxymethylation is seen at regulatory regions in pancreatic cancer and regulates oncogenic pathways. Genome Res. 2017 Nov;27(11):1830–1842.
[57] Stead LF, Verhaak RGW. Doomed from the TERT? A Two-Stage Model of Tumorigenesis in IDH-Wild-Type Glioblastoma. Cancer Cell. 2019 Apr 15;35(4):542–544.
[58] Weintraub AS, Li CH, Zamudio AV, et al. YY1 Is a Structural Regulator of Enhancer-Promoter Loops. Cell. 2017 Dec 14;171(7):1573–1588 e28.
[59] Zeid R, Lawlor MA, Poon E, et al. Enhancer invasion shapes MYCN-dependent transcriptional amplification in neuroblastoma. Nat Genet. 2018 Apr;50(4):515–523.
[60] Kohnken R, Wen J, Mundy-Bosse B, et al. Diminished microRNA-29b level is associated with BRD4-mediated activation of oncogenes in cutaneous T-cell lymphoma. Blood. 2018 Feb 15;131(7):771–781.
[61] Liu Y, Li Y, Liu S, et al. NK Cells Mediate Synergistic Antitumor Effects of Combined Inhibition of HDAC6 and BET in a SCLC Preclinical Model. Cancer Res. 2018 Jul 1;78(13):3709–3717.
[62] Michel BC, D'Avino AR, Cassel SH, et al. A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation. Nat Cell Biol. 2018 Dec;20(12):1410–1420.
[63] Su R, Dong L, Li C, et al. R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. Cell. 2018 Jan 11;172(1–2):90–105 e23.
[64] Xu L, Chen Y, Mayakonda A, et al. Targetable BET proteins- and E2F1-dependent transcriptional program maintains the malignancy of glioblastoma. Proc Natl Acad Sci U S A. 2018 May 29;115(22):E5086–E5095.
[65] Bandopadhayay P, Piccioni F, O'Rourke R, et al. Neuronal differentiation and cell-cycle programs mediate response to BET-bromodomain inhibition in MYC-driven medulloblastoma. Nat Commun. 2019 Jun 3;10(1):2400.
[66] Hemming ML, Lawlor MA, Andersen JL, et al. Enhancer Domains in Gastrointestinal Stromal Tumor Regulate KIT Expression and Are Targetable by BET Bromodomain Inhibition. Cancer Res. 2019 Mar 1;79(5):994–1009.
[67] MillerAL, FehlingSC, GarciaPL, et al. EBioMedicine. In: The BET inhibitor JQ1 attenuates double-strand break repair and sensitizes models ofpancreatic ductal adenocarcinoma to PARP inhibitors. May,2019;44:419–430.Epub 2019 May22doi:10.1016/j.ebiom.2019.05.035.
[68] Sdelci S, Rendeiro AF, Rathert P, et al. MTHFD1 interaction with BRD4 links folate metabolism to transcriptional regulation. Nat Genet. 2019 Jun;51(6):990–998.
[69] FierceBiotech. Jay Bradner - Novartis: FiercePharma; 2018 [cited 2019 Jun 6]. Available from: https://www.fiercebiotech.com/special-report/jay-bradner-novartis
[70] Novartis. James (Jay) Bradner, President of Novartis Institutes for Biomedical Research (NIBR) Cambridge, Massachusetts, USA: Novartis; 2019 [cited 2019 Jun 9]. Available from: https://www.fiercebiotech.com/special-report/jay-bradner-novartis

[71] Murphy SK, Itchon-Ramos N, Visco Z, et al. Epigenetics. In: Cannabinoid exposure and altered DNA methylation in rat and human sperm. Epigenetics. 2018;13(12):1208–1221..
[72] Liu S, Kwon M, Mannino M, et al. Nuclear envelope assembly defects link mitotic errors to chromothripsis. Nature. 2018 Sep;561(7724):551–555.
[73] Reece AS. Chronic toxicology of cannabis. Clin Toxicol (Phila). 2009 Jul;47(6):517–524.
[74] Callaghan RC, Allebeck P, Akre O, et al. Cannabis Use and Incidence of Testicular Cancer: A 42-Year Follow-up of Swedish Men between 1970 and 2011. Cancer Epidemiol Biomarkers Prev. 2017 Nov;26 (11):1644–1652.
[75] Daling JR, Doody DR, Sun X, et al. Association of marijuana use and the incidence of testicular germ cell tumors. Cancer. 2009 Mar 15;115(6):1215–1223.
[76] Lacson JC, Carroll JD, Tuazon E, et al. Population-based case-control study of recreational drug use and testis cancer risk confirms an association between marijuana use and nonseminoma risk. Cancer. 2012 Nov 1;118(21):5374–5383.
[77] Trabert B, Sigurdson AJ, Sweeney AM, et al. Marijuana use and testicular germ cell tumors. Cancer. 2011 Feb 15;117(4):848–853.
[78] Gurney J, Shaw C, Stanley J, et al. Cannabis exposure and risk of testicular cancer: a systematic review and meta-analysis. BMC Cancer. 2015 11;Nov(15):897.
[79] Sweet-Cordero EA, Biegel JA. The genomic landscape of pediatric cancers: implications for diagnosis and treatment. Science. 2019 Mar 15;363 (6432):1170–1175.
[80] Lam CG, Howard SC, Bouffet E, et al. Science and health for all children with cancer. Science. 2019 Mar 15;363(6432):1182–1186.
[81] David AL, Holloway A, Thomasson L, et al. A case-control study of maternal periconceptual and pregnancy recreational drug use and fetal malformation using hair analysis. PLoS One. 2014;9(10): e111038.
[82] Draper ES, Rankin J, Tonks AM, et al. Recreational drug use: a major risk factor for gastroschisis?. Am J Epidemiol. 2008 Feb 15;167(4):485–491.
[83] Forrester MB, Merz RD. Risk of selected birth defects with prenatal illicit drug use, Hawaii, 1986-2002. J Toxicol Environ Health. 2007 Jan;70(1):7–18.
[84] Skarsgard ED, Meaney C, Bassil K, et al. Maternal risk factors for gastroschisis in Canada. Birth Defects Res A Clin Mol Teratol. 2015 Feb;103(2):111–118.
[85] Torfs CP, Velie EM, Oechsli FW, et al. A population-based study of gastroschisis: demographic, pregnancy, and lifestyle risk factors. Teratology. 1994 Jul;50(1):44–53.
[86] van Gelder MM, Reefhuis J, Caton AR, et al. Maternal periconceptional illicit drug use and the risk of congenital malformations. Epidemiology. 2009 Jan;20 (1):60–66.
[87] Werler MM, Sheehan JE, Mitchell AA. Association of vasoconstrictive exposures with risks of gastroschisis and small intestinal atresia. Epidemiology. 2003 May;14(3):349–354.
[88] Volkow ND, Compton WM, Weiss SR. Adverse health effects of marijuana use. N Engl J Med. 2014 Aug 28;371(9):879.
[89] Bindukumar B, Mahajan SD, Reynolds JL, et al. Genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes. Brain Res. 2008 Jan;29(1191):1–11.
[90] Boa-AmponsemO, ZhangC, MukhopadhyayS, et al. Birth Defects Res. In: Ethanol and cannabinoids interact to alter behavior in a zebrafish fetal alcohol spectrum disorder model. Birth Defects Research. 2019 Jan 16. [Epub ahead of print].doi:10.1002/bdr2.1458.
[91] Subbanna S, Nagre NN, Shivakumar M, et al. CB1R-Mediated Activation of Caspase-3 Causes Epigenetic and Neurobehavioral Abnormalities in Postnatal Ethanol-Exposed Mice. Front Mol Neurosci. 2018;11:45.
[92] Nagre NN, Subbanna S, Shivakumar M, et al. CB1-receptor knockout neonatal mice are protected against ethanol-induced impairments of DNMT1, DNMT3A, and DNA methylation. J Neurochem. 2015 Feb;132 (4):429–442.
[93] Banik A, Kandilya D, Ramya S, et al. Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes (Basel). 2017 May 24;8(6). doi:10.3390/genes8060150.
[94] Chater-Diehl EJ, Laufer BI, Singh SM. Changes to histone modifications following prenatal alcohol exposure: an emerging picture. Alcohol. 2017 May;60:41–52.
[95] Lunde ER, Washburn SE, Golding MC, et al. Alcohol-Induced Developmental Origins of Adult-Onset Diseases. Alcohol Clin Exp Res. 2016 Jul;40(7):1403–1414.
[96] Veazey KJ, Wang H, Bedi YS, et al. Disconnect between alcohol-induced alterations in chromatin structure and gene transcription in a mouse embryonic stem cell model of exposure. Alcohol. 2017 May;60:121–133. (Fayetteville, NY.
[97] Fauci AS, Braunwald E, Kapser DL, et al. editors. Harrison's Principles of Internal Medicine, 17th Edition. 17th Edition. New York: McGraw Hill; 2008.
[98] Smith AM, Fried PA, Hogan MJ, et al. Effects of prenatal marijuana on visuospatial working memory: an fMRI study in young adults. Neurotoxicol Teratol. 2006 Mar-Apr;28(2):286–295.
[99] Smith AM, Longo CA, Fried PA, et al. Effects of marijuana on visuospatial working memory: an fMRI study in young adults. Psychopharmacology (Berl). 2010 Jun;210(3):429–438.
[100] Smith AM, Mioduszewski O, Hatchard T, et al. Prenatal marijuana exposure impacts executive functioning into young adulthood: an fMRI study. Neurotoxicol Teratol. 2016;58:53–59.

[101] Brents L. Correlates and consequences of Prenatal Cannabis Exposure (PCE): identifying and Characterizing Vulnerable Maternal Populations and Determining Outcomes in Exposed Offspring. In: Preedy VR, editor. Handbook of Cannabis and Related Pathologies: biology, Pharmacology, Diagnosis and Treatment. Vol. 1. London: Academic Press; 2017. p. 160–170.

[102] National Birth Defects Prevention Network. National Birth Defects Prevention Network Houston, Texas, USA: National Birth Defects Prevention Network; 2018 [cited 2018 Jul 15]. Available from: https://www.nbdpn.org/ar.php

[103] Nevison C, Blaxill M, Zahorodny W. California Autism Prevalence Trends from 1931 to 2014 and Comparison to National ASD Data from IDEA and ADDM. J Autism Dev Disord. 2018 Dec;48(12):4103–4117.

[104] Public Health Agency of Canada, Health Canada. Congenital Anomalies in Canada, 2013. A Perinatal Health Surveillance Report. In: Public Health Agency of Canada, Health Canada. Ottawa: Health Canada; 2013. p. 1–119.

[105] Statistics Canada. National Cannabis Survey, Second Quarter, 2018 Ottawa Canada: Statistics Canada; 2018 [cited 2019 Mar 17]. Available from: https://www.facebook.com/StatisticsCanada/posts/1636405843137586:0

[106] Colorado: Department of Public Health and the Environment. Colorado Responds to Children with Special Needs - Birth Defect Data, Colorado. In: Environment CDoPHat. Denver Colorado, USA: Colorado: Department of Public Health and the Environment; 2018. http://www.chd.dphe.state.co.us/cohid/

[107] Substance Abuse and Mental Health Administration, Department of Health and Human Services, United States Government. National Survey of Drug Use and Health 2018, NSDUH Bethesda, Maryland: Substance Abuse and Mental Health Administration, Department of Health and Human Services, United States Government,; 2018 [cited 2 June, 2018]. Available from: https://www.samhsa.gov/data/all-reports

[108] Reece AS, Hulse GK. Effect of Cannabis Legalization on US Autism Incidence and Medium Term Projections. Clin Pediatr Open Access. 2019;4(2). In Press.

[109] Reece AS, Hulse GK Epidemiological Associations of Various Substances and Multiple Cannabinoids with Autism in USA. Submitted to Pediatrics. 2019.

[110] Willsher K Baby arm defects prompt nationwide investigation in France. Guardian. 2018. https://www.theguardian.com/world/2018/oct/31/baby-arm-defects-prompt-nationwide-investigation-france

[111] Agence France-Presse in Paris. France to investigate cause of upper limb defects in babies. The Guardian. 2018 [cited 2018 Nov 3][https://www.theguardian.com/world/2018/oct/21/france-to-investigate-cause-of-upper-limb-defects-in-babies

[112] Huang H, Liu S, Kornberg TB. Glutamate signaling at cytoneme synapses. Science. 2019 Mar 1;363(6430):948–955.

[113] Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet. 2000 Apr;16(4):168–174.

[114] Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. 2012 Sep;143(3):550–563.

[115] Issa JP, Ahuja N, Toyota M, et al. Accelerated age-related CpG island methylation in ulcerative colitis. Cancer Res. 2001 May 1;61(9):3573–3577.

[116] Vaz M, Hwang SY, Kagiampakis I, et al. Chronic Cigarette Smoke-Induced Epigenomic Changes Precede Sensitization of Bronchial Epithelial Cells to Single-Step Transformation by KRAS Mutations. Cancer Cell. 2017 Sep 11;32(3):360–376 e6.

[117] O'Hagan HM, Wang W, Sen S, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. Cancer Cell. 2011 Nov 15;20(5):606–619.

[118] Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004 Dec;4(12):988–993.

[119] Niwa T, Ushijima T. Induction of epigenetic alterations by chronic inflammation and its significance on carcinogenesis. Adv Genet. 2010;71:41–56.

[120] Maegawa S, Gough SM, Watanabe-Okochi N, et al. Age-related epigenetic drift in the pathogenesis of MDS and AML. Genome Res. 2014 Apr;24(4):580–591.

[121] Reece AS, Norman A, Hulse GK. Cannabis Exposure as an Interactive Cardiovascular Risk Factor and Accelerant of Organismal Ageing – A Longitudinal Study. BMJ Open. 2016 Nov 7;6(11):e011891. doi:10.1136/bmjopen-2016-01189.

[122] De Cecco M, Ito T, Petrashen AP, et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature. 2019 Feb;566(7742):73–78.

[123] Andriani GA, Almeida VP, Faggioli F, et al. Whole Chromosome Instability induces senescence and promotes SASP. Sci Rep. 2016 Oct;12(6):35218.

[124] Cannizzo ES, Clement CC, Sahu R, et al. Oxidative stress, inflamm-aging and immunosenescence. J Proteomics. 2011 Oct 19;74(11):2313–2323.

[125] Salminen A, Huuskonen J, Ojala J, et al. Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev. 2008 Apr;7(2):83–105.

[126] Reece AS, Norman A, Hulse GK. Cannabis Exposure as an Interactive Cardiovascular Risk Factor and Accelerant of Organismal Ageing – A Longitudinal Study. BMJ - Open. 2016;6(11):e011891–e011900.

[127] Zimmerman AM, Zimmerman S, Raj AY, et al. Effects of Cannabinoids on spermatogenesis in mice. In: Nahas GG, Sutin KM, Harvey DJeditors. Marihuana and medicine. Totowa, N.J, USA: Humana Press; 1999. p. 347–358.

[128] Zimmerman AM, Raj AY. Influence of cannabinoids on somatic cells in vivo. Pharmacology. 1980;21 (4):277–287.
[129] Zimmerman S, Zimmerman AM. Genetic effects of marijuana. Int J Addict. 1990;25(1A):19–33.
[130] Zimmerman AM, Bruce WR, Zimmerman S. Effects of cannabinoids on sperm morphology. Pharmacology. 1979;18(3):143–148.
[131] Hembree WC 3rd, Nahas GG, Zeidenberg P, et al. Changes in human spermatozoa associated with high dose marihuana smoking. Adv Biosci. 1978 Jul 22-23;22-23:429–439.
[132] Huang HFSNGG, Hembree WC. Effects of marijuana inhalation on spermatogenesis of the rat. In: Nahas GGSKM, Harvey DJ, Agurell S, editors. Marijuana and medicine. Totowa, New Jersey: Humana Press; 1999. p. 359–366.
[133] Morishima A. Effects of cannabis and natural cannabinoids on chromosomes and ova. NIDA Res Monogr. 1984;44:25–45.
[134] Mihalas BP, Redgrove KA, McLaughlin EA, et al. Molecular Mechanisms Responsible for Increased Vulnerability of the Ageing Oocyte to Oxidative Damage. Oxid Med Cell Longev. 2017;2017:4015874.
[135] Di Nisio V, Rossi G, Palmerini MG, et al. Increased rounds of gonadotropin stimulation have side effects on mouse fallopian tubes and oocytes. Reproduction. 2018 Mar;155(3):245–250.
[136] Hughes SE, Miller DE, Miller AL, et al. Female Meiosis: synapsis, Recombination, and Segregation in Drosophila melanogaster. Genetics. 2018 Mar;208 (3):875–908.
[137] Stefanis CN, Issidorides MR. Cellular Effects of Chronic Cannabis Use in Man. In: Nahas GG, editor. Marihuana: chemistry, Biochemistry and Cellular Effects. Vol. 1. New York: Springer; 1976. p. 533–550.
[138] Reece AS, Hulse GK. Chromothripsis and epigenomics complete causality criteria for cannabis- and addiction-connected carcinogenicity, congenital toxicity and heritable genotoxicity. Mutat Res. 2016 Jul;789:15–25.
[139] Piatti E, Rizzi R, Re F, et al. Genotoxicity of heroin and cannabinoids in humans. Pharmacol Res. 1989; Nov-Dec;21(Suppl 1):59–60.
[140] Van Went GF. Mutagenicity testing of 3 hallucinogens: LSD, psilocybin and delta 9-THC, using the micronucleus test. Experientia. 1978 Mar 15;34 (3):324–325.
[141] Pellestor F. Chromoanagenesis: cataclysms behind complex chromosomal rearrangements. Mol Cytogenet. 2019;12:6.
[142] Kloosterman WP, Guryev V, van Roosmalen M, et al. Chromothripsis as a mechanism driving complex de novo structural rearrangements in the germline. Hum Mol Genet. 2011 May 15;20(10):1916–1924.
[143] Gluck S, Guey B, Gulen MF, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. Nat Cell Biol. 2017 Sep;19(9):1061–1070.
[144] Mackenzie KJ, Carroll P, Martin CA, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature. 2017 Aug 24;548 (7668):461–465.
[145] Yang H, Wang H, Ren J, et al. cGAS is essential for cellular senescence. Proc Natl Acad Sci U S A. 2017 Jun 6;114(23):E4612–E4620.
[146] Ablasser A, Chen ZJ. cGAS in action: expanding roles in immunity and inflammation. Science. 2019;363 (6431):eaat8657.
[147] Bakhoum SF, Ngo B, Laughney AM, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. Nature. 2018 Jan 25;553 (7689):467–472.
[148] Van Gelder MMHJ, Donders ART, Devine O, et al. Using bayesian models to assess the effects of under-reporting of cannabis use on the association with birth defects, national birth defects prevention study, 1997-2005 [Article]. Paediatr Perinat Epidemiol. 2014;28(5):424–433.
[149] Van Gelder MMHJ, Reefhuis J, Caton AR, et al. Maternal periconceptional illicit drug use and the risk of congenital malformations. Epidemiology. 2009 January;20(1):60–66.
[150] Liu W, Komiya Y, Mezzacappa C, et al. MIM regulates vertebrate neural tube closure. Development. 2011 May;138(10):2035–2047.
[151] Harper KL, Sosa MS, Entenberg D, et al. Mechanism of early dissemination and metastasis in Her2(+) mammary cancer. Nature. 2016 Dec 22;540(7634):588–592.
[152] Janda CY, Dang LT, You C, et al. Surrogate Wnt agonists that phenocopy canonical Wnt and beta-catenin signalling. Nature. 2017 May 11;545(7653):234–237.
[153] Tammela T, Sanchez-Rivera FJ, Cetinbas NM, et al. A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. Nature. 2017 May 18;545(7654):355–359.
[154] Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. Nature. 2017 Jul 19;547(7663):311–317.
[155] Szenker-Ravi E, Altunoglu U, Leushacke M, et al. RSPO2 inhibition of RNF43 and ZNRF3 governs limb development independently of LGR4/5/6. Nature. 2018 May;557(7706):564–569.
[156] Liyanage VRB, Olson CO, Zachariah RM, et al. DNA Methylation Contributes to the Differential Expression Levels of Mecp2 in Male Mice Neurons and Astrocytes. Int J Mol Sci. 2019 Apr 14;20(8). pii: E1845. doi: 10.3390/ijms20081845.
[157] Kuehner JN, Bruggeman EC, Wen Z, et al. Epigenetic Regulations in Neuropsychiatric Disorders. Front Genet. 2019;10:268.

[158] Garg P, Sharp AJ. Screening for rare epigenetic variations in autism and schizophrenia. Hum Mutat. 2019 Mar 21. doi:10.1002/humu.23740. [Epub ahead of print].
[159] Bend EG, Aref-Eshghi E, Everman DB, et al. Gene domain-specific DNA methylation episignatures highlight distinct molecular entities of ADNP syndrome. Clin Epigenetics. 2019 Apr 27;11(1):64.
[160] Alex AM, Saradalekshmi KR, Shilen N, et al. IUBMB Life. In: Genetic association of DNMT variants can play a critical role in defining the methylation patterns in autism. IUBMB Life. 2019 Jul;71(7):901–907. doi:10.1002/iub.2021. Epub 2019 Feb 20.
[161] Krol KM, Puglia MH, Morris JP, et al. Epigenetic modification of the oxytocin receptor gene is associated with emotion processing in the infant brain. Dev Cogn Neurosci. 2019;37:100648.
[162] Russo C, Ferk F, Misik M, et al. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. Arch Toxicol. 2019 Jan;93(1):179–188. Epub 2018 Oct 19.doi: 10.1007/s00204-018-2322-9.
[163] Zimmerman AM, Zimmerman S, Raj AY. Effects of Cannabinoids on Spermatogensis in Mice. In: Nahas GG, Sutin KM, Harvey DJ, et al., editors. Marijuana and Medicine. Vol. 1. Totowa, New York: Humana Press; 1999. p. 347–358.
[164] Chiu P, Karler R, Craven C, et al. The influence of delta9-tetrahydrocannabinol, cannabinol and cannabidiol on tissue oxygen consumption. Res Commun Chem Pathol Pharmacol. 1975 Oct;12(2):267–286.
[165] Fisar Z, Singh N, Hroudova J. Cannabinoid-induced changes in respiration of brain mitochondria. Toxicol Lett. 2014 Nov 18;231(1):62–71.
[166] Hayakawa K, Mishima K, Hazekawa M, et al. Cannabidiol potentiates pharmacological effects of Delta (9)-tetrahydrocannabinol via CB(1) receptor-dependent mechanism. Brain Res. 2008 Jan 10;1188:157–164.
[167] Mato S, Victoria Sanchez-Gomez M, Matute C. Cannabidiol induces intracellular calcium elevation and cytotoxicity in oligodendrocytes. Glia. 2010 Nov 1;58 (14):1739–1747.
[168] Silvestri C, Di Marzo V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab. 2013 Apr 2;17(4):475–490.
[169] Rimmerman N, Ben-Hail D, Porat Z, et al. Direct modulation of the outer mitochondrial membrane channel, voltage-dependent anion channel 1 (VDAC1) by cannabidiol: a novel mechanism for cannabinoid-induced cell death. Cell Death Dis. 2013 Dec 5;4:e949.
[170] Ryan D, Drysdale AJ, Lafourcade C, et al. Cannabidiol targets mitochondria to regulate intracellular Ca2+ levels. J Neurosci. 2009 Feb 18;29(7):2053–2063.
[171] Singh N, Hroudova J, Fisar Z. Cannabinoid-Induced Changes in the Activity of Electron Transport Chain Complexes of Brain Mitochondria. J Mol Neurosci. 2015 Aug;56(4):926–931.
[172] Alhamoruni A, Lee AC, Wright KL, et al. Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability. J Pharmacol Exp Ther. 2010 Oct;335(1):92–102.
[173] Da Silva JA, Biagioni AF, Almada RC, et al. Dissociation between the panicolytic effect of cannabidiol microinjected into the substantia nigra, pars reticulata, and fear-induced antinociception elicited by bicuculline administration in deep layers of the superior colliculus: the role of CB1-cannabinoid receptor in the ventral mesencephalon. Eur J Pharmacol. 2015 Jul 5;758:153–163.
[174] Hwang YS, Kim YJ, Kim MO, et al. Cannabidiol upregulates melanogenesis through CB1 dependent pathway by activating p38 MAPK and p42/44 MAPK. Chem Biol Interact. 2017 Aug 1;273:107–114.
[175] Laprairie RB, Bagher AM, Kelly ME, et al. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br J Pharmacol. 2015 Oct;172(20):4790–4805.
[176] Sartim AG, Guimaraes FS, Joca SR. Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex-Possible involvement of 5-HT1A and CB1 receptors. Behav Brain Res. 2016 Apr 15;303:218–227.
[177] Stanley CP, Hind WH, Tufarelli C, et al. Cannabidiol causes endothelium-dependent vasorelaxation of human mesenteric arteries via CB1 activation. Cardiovasc Res. 2015 Sep 1;107(4):568–578.
[178] Stern CAJ, Da Silva TR, Raymundi AM, et al. Cannabidiol disrupts the consolidation of specific and generalized fear memories via dorsal hippocampus CB1 and CB2 receptors. Neuropharmacology. 2017;125:220–230.
[179] Pucci M, Rapino C, Di Francesco A, et al. Epigenetic control of skin differentiation genes by phytocannabinoids. Br J Pharmacol. 2013 Oct;170 (3):581–591.
[180] De Filippis D, Esposito G, Cirillo C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. PLoS One. 2011;6(12):e28159.
[181] Esposito G, Scuderi C, Valenza M, et al. Cannabidiol reduces Abeta-induced neuroinflammation and promotes hippocampal neurogenesis through PPARgamma involvement. PLoS One. 2011;6(12):e28668.
[182] Hegde VL, Singh UP, Nagarkatti PS, et al. Critical Role of Mast Cells and Peroxisome Proliferator-Activated Receptor gamma in the Induction of Myeloid-Derived Suppressor Cells by Marijuana Cannabidiol In Vivo. J Immunol. 2015 Jun 1;194(11):5211–5222.
[183] Hind WH, England TJ, O'Sullivan SE. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARgamma and 5-HT1A receptors. Br J Pharmacol. 2016 Mar;173 (5):815–825.
[184] O'Sullivan SE, Kendall DA. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. Immunobiology. 2010 Aug;215(8):611–616.

[185] O'Sullivan SE, Sun Y, Bennett AJ, et al. Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol. 2009 Jun 10;612(1–3):61–68.
[186] Ramer R, Heinemann K, Merkord J, et al. COX-2 and PPAR-gamma confer cannabidiol-induced apoptosis of human lung cancer cells. Mol Cancer Ther. 2013 Jan;12 (1):69–82.
[187] Scuderi C, Steardo L, Esposito G. Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5YAPP + cells through PPARgamma involvement. Phytother Res. 2014 Jul;28(7):1007–1013.
[188] Tyagi S, Gupta P, Saini AS, et al. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J Adv Pharm Technol Res. 2011 Oct;2(4):236–240.
[189] Cutando L, Maldonado R, Ozaita A. Microglial Activation and Cannabis Exposure. In: Preedy V. editor. Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis and Treatment. New York: Academic Press; 2017:401–12.
[190] Leos-Toro C, Reid JL, Madill CL, et al. Cannabis in Canada - Tobacco Use in Canada: patterns and Trends, 2017 Edition, Special Supplement. In: PROPEL, Centre for Population Health Impact, Waterloo University, editors. Cannabis in Canada: patterns and Trends, 2017. Waterloo, Ontario: University of Waterloo; 2017. p. 1–23.
[191] Public Health Agency of Canada. Congenital Anomalies in Canada, 2013. A Perinatal Health Surveillance Report In: public Health Agency of Canada HC, editor. Ottawa: Health Canada; 2013. p. 1–115.
[192] Arbour L, Gilpin C, Millor-Roy V, et al. Heart defects and other malformations in the Inuit in Canada: a baseline study. Int J Circumpolar Health. 2004 Sep;63(3):251–266.
[193] Pacher P, Steffens S, Hasko G, et al. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. Nat Rev Cardiol. 2018 Mar;15(3):151–166.
[194] Frampton G, Coufal M, Li H, et al. Opposing actions of endocannabinoids on cholangiocarcinoma growth is via the differential activation of Notch signaling. Exp Cell Res. 2010 May 15;316(9):1465–1478.
[195] Newton CA, Chou PJ, Perkins I, et al. CB(1) and CB(2) cannabinoid receptors mediate different aspects of delta-9-tetrahydrocannabinol (THC)-induced T helper cell shift following immune activation by Legionella pneumophila infection. J Neuroimmune Pharmacol. 2009 Mar;4(1):92–102.
[196] Tanveer R, Gowran A, Noonan J, et al. The endocannabinoid, anandamide, augments Notch-1 signaling in cultured cortical neurons exposed to amyloid-beta and in the cortex of aged rats. J Biol Chem. 2012 Oct 5;287(41):34709–34721.
[197] BM C. Human Embryology and Developmental Biology. Vol. 1. Philadelphia: Elsevier; 2014.
[198] Basu M, Zhu JY, LaHaye S, et al. Epigenetic mechanisms underlying maternal diabetes-associated risk of congenital heart disease. In: J. Clin. Iinvest. Insight. 2017 Oct 19;2(20). pii: 95085. doi:10.1172/jci.insight.95085.
[199] Chatterjee R, Law S. Epigenetic and microenvironmental alterations in bone marrow associated with ROS in experimental aplastic anemia. Eur J Cell Biol. 2018 Jan;97(1):32–43.
[200] Kwon H, Song K, Han C, et al. Epigenetic Silencing of miRNA-34a in Human Cholangiocarcinoma via EZH2 and DNA Methylation: impact on Regulation of Notch Pathway. Am J Pathol. 2017 Oct;187(10):2288–2299.
[201] Jin L, Vu T, Yuan G, et al. STRAP Promotes Stemness of Human Colorectal Cancer via Epigenetic Regulation of the NOTCH Pathway. Cancer Res. 2017 Oct 15;77 (20):5464–5478.
[202] Katoh M. Canonical and non-canonical WNT signaling in cancer stem cells and their niches: cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review). Int J Oncol. 2017 Nov;51(5):1357–1369.
[203] Kuang SQ, Fang Z, Zweidler-McKay PA, et al. Epigenetic inactivation of Notch-Hes pathway in human B-cell acute lymphoblastic leukemia. PLoS One. 2013;8(4):e61807.
[204] Lian H, Jia X, Shi N, et al. Notch signaling promotes serrated neoplasia pathway in colorectal cancer through epigenetic modification of EPHB2 and EPHB4. Cancer Manag Res. 2018;10:6129–6141.
[205] Geber WF, Schramm LC. Teratogenicity of marihuana extract as influenced by plant origin and seasonal variation. Arch Int Pharmacodyn Ther. 1969 Jan;177 (1):224–230.
[206] Graham JDP. Cannabis and Health. In: Graham JDP, editor. Cannabis and Health. Vol. 1. 1 ed ed. London, New York, San Francisco: Academic Press; 1976. p. 271–320.