Review Article

ROLE OF PHYSICAL EXERCISE IN THE REGULATION OF EPIGENETIC MECHANISMS IN INFLAMMATION, CANCER, NEURODEGENERATIVE DISEASES AND AGING PROCESS

Martina Ferioli[1], Giorgio Zauli[1], Patrizia Maiorano[2], Daniela Milani[1], Prisco Mirandola[3] and Luca M. Neri[1*]

[1]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy;

[2]Department of Medical Biotechnology, University of Siena, Siena, Italy;

[3]Department of Medicine and Surgery, University of Parma, Parma, Italy

*Correspondence to: **Luca M. Neri.** Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Italy. Phone:+39-0532-455940, Fax: +39-0532-207351, E-mail: luca.neri@unife.it

Keywords: epigenetics, physical exercise, inflammation, cancer, aging.

Total number of text Figures and Tables : 1 (1 Table)

Abstract

The genetic heritage for decades has been considered responding only to gene promoters or suppressors, with specific roles for oncogenes or tumour suppressor genes.

Epigenetics is progressively attracting an increasing interest since it has been demonstrated the capacity of these regulatory processes to regulate gene expression without modifying gene sequence.

Several factors may influence epigenetics, such as lifestyles including food selection. A role for physical exercise is emerging in epigenetic regulation of gene expression.

In this review we resume physiological and pathological implications of epigenetic modification induced by physical activity. Inflammation and cancer mechanisms, immune system, central nervous system and the aging process receive benefits due to physical activity through epigenetic mechanisms.

Thus the modulation of epigenetic processes by physical exercise positively influences prevention, development and the course of inflammatory and cancer diseases, as well as neurodegenerative illnesses.

This growing field of studies gives rise to a new role for physical activity as an option in prevention strategies and to integrate pharmacological therapeutic treatments.

INTRODUCTION

The word “epigenetic” refers to regulatory processes that influence gene expression without changing the DNA sequence (Donohoe and Bultman, 2012). Many of those have been identified and included in the definition of epigenetic. It’s possible to summarize these various processes in three categories: DNA modifications that do not affect the base sequence (such as DNA CpG methylation), post translational modifications of histone proteins (such as deacetylation and/or methylation of histone proteins) and expression of micro-RNA (mi-RNA) (Ellis et al., 2009; Mann, 2014).

Physical activity (PA) is defined as any bodily movement produced by skeletal muscles that results in energy expenditure, which may refer to everyday life activity, exercise that includes prearranged, deliberate and repetitive activity and grassroots sports and competitive sports (Condello et al., 2016). Physical activity and healthy nutrition are considered key points for the prevention of chronic and neoplastic diseases (Barone et al., 2018).

It is known that exercise is a safe, non-pharmacological and cost-effective strategy to prevent the development of most diseases, to slow down their progression, but also to provide benefits in patients affected by chronic diseases (Ferioli et al., 2018).

Does physical activity influence epigenetic? And if yes, could physical activity modulate epigenetic mechanisms to prevent the development of physiological alterations that lead to diseases? Could physical activity act as a “therapy” to restore epigenetic “balance” in cancer and in non-neoplastic disease? In the next paragraphs we try to develop answers to these questions.

EPIGENETIC REGULATORY PROCESSES

DNA CpG methylation

One process is DNA CpG methylation that consists in the addition of a methyl group (CH3) on cytosine followed by guanine base within the DNA. This process is catalysed by DNA methyltransferase enzymes (DNMTs) (Ghosh et al., 2017). The genome regions rich in CpG

dinucleotides are called “CpG islands” and they are often localized in the gene regulatory promoter regions. When the “CpG islands” are methylated, proteins that bind DNA called methylated DNA-binding proteins (MDBPs) cover the promoter region, preventing the binding between DNA and transcriptional factors or inducing modification of chromatin structure thus resulting in the inhibition of gene expression (Jang et al., 2017; Kim et al., 2009). The DNA methylation (and consequently the silencing) of the tumour-suppressor genes is one of the most studied epigenetic mechanism in cancer (Li et al., 2018). The DNA methylation of other dinucleotides (such as cytosines followed by adenine or thymine) is called “Non-CpG methylation” and has a role in embryonic stem cells (Laurent et al., 2010).

The methylation of DNA is a stable but reversible process. DNA demethylation can be a passive process that consists in loss of 5-methylcytosine from newly synthesised DNA strands during the following replication rounds. Otherwise, active DNA demethylation involves enzymes that may remove the methyl group from 5-methylcytosine. DNA hydroxy-methylation, the initial process of DNA de-methylation, is mediated by Ten-Eleven Translocation (TET) enzymes and determines the oxidation of methyl-CpG to generate hydroxymethyl-CpG. Thus, the result of DNA hydroxymethylation is the stimulation of gene transcription (Kohli and Zhang, 2013; Tahiliani et al., 2009).

Post translational modifications (PTMs) of histone proteins

A second epigenetic mechanism that directly influences gene expression is the post translational modifications (PTMs) of histone proteins.

The assembling of DNA and histone proteins is called chromatin. The structure of chromatin is organized in histone octamers surrounded by a double strand of DNA that wraps 2,5 times around these histone cores, forming the “beads on a string” arrangement. Each bead is called “nucleosome” and each octamer is composed of a pair of histones: H2A, H2B, H3 and H4. (Eberharter and Becker, 2002). Nucleosome formation is guaranteed by opposite charges of histone proteins (H4 are

positive while the surface of H2A histone fold domains are negative) (Kurdistani and Grunstein, 2003).

PTMs of histones include histone acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation (Roostaee et al., 2016). These modifications occur predominantly on the accessible histone tails (Rothbart and Strahl, 2014).

The process of histone acetylation is mediated by histone acetyltransferases (HATs) (Shafabakhsh et al., 2018), which catalyse the addition of an acetyl group (COCH3) from acetyl coenzyme to the NH3+ groups of lysine residues. On the other hand, histone deacetylation consists in the hydrolytic removal of acetyl groups from histone, driven by histone deacetylases (HDACs) (Vecera et al., 2018). Acetylation changes the overall charge of the histone that turns into neutral: the nucleosome structure becomes weaker, resulting in an increased accessibility to the DNA for transcription factors. Consequently, acetylation facilitates gene transcription while deacetylation inhibits gene transcription (Jahan et al., 2018; Zentner and Henikoff, 2013). The deregulation of histone acetylation has been associated with tumour development and cancer progression (Sigalotti et al., 2007). In particular, the mutations of genes that encode for HDACs have been associated with tumorigenesis since they influence the transcription of genes involved in cell-cycle regulation and apoptosis (Liu et al., 2017). This is why HDACs is considered a pharmacological target for anticancer agents. At present, four HDAC inhibitors (HDACIs) have been approved by the US Food and Drug Administration (FDA) as anti-cancer treatment, one for peripheral T-cell lymphoma (PTCL), one other for multiple myeloma, and two for cutaneous T-cell lymphoma (Zhang et al., 2018).

Histone methylation occurs by enzymatic addition of one, two or three methyl groups from S-adenosyl-L-methionine to lysine or arginine amino acids. The addition of the methyl group may be a stably maintained or a reversible phenomenon through arginine and lysine methyltransferases (KMTs) and demethylases (KDMs). Histone methylation does not change histone charge and does not interfere with DNA association, ~~but~~ in some cases it promotes transcription or in other cases is

associated with repression of transcription by affecting the binding of proteins called histone readers (Daskalaki et al., 2018; Greer and Shi, 2012). An imbalance of histone methylation has been associated with the aging process, intellectual disability syndromes and cancer (Greer and Shi, 2012; McCauley and Dang, 2014). In particular an aberrant global histone lysine methylation level was found in several cancer cell lines (Chi et al., 2010). This is why methylation/demethylation histones enzymes recently become an interesting target for cancer therapy, with positive results in selective cancer cell killing in vitro (McGrath and Trojer, 2015).

Histone phosphorylation is defined as the attachment of a phosphoryl group on serine, threonine or tyrosine residues of the histone code by protein-kinases. Histone phosphorylation is involved in DNA damage response, but it also influences DNA accessibility of transcription regulatory complexes: phosphorylation has been associated with gene expression, especially of proliferation genes (Brehove et al., 2015; Rossetto et al., 2012). Several studies in recent years focused on histone H1 alterations in cancer, both as potential biomarker and as a driver of modification in cancer (Scaffidi, 2016); for example Histone H1 phosphorylation was related to bladder cancer grade (Telu et al., 2013). Harshman et al. found that in breast cancer the global level of histone H1 phosphorylation changes in response to extracellular therapeutic stimulation in vitro, suggesting that this phosphorylation could become a substantial biomarker of patient response to antineoplastic agents (Harshman et al., 2014).

Histone ubiquitylation is the addition of a molecule of ubiquitin to lysine residues of histones. This process may result in proteasome mediated degradation (Cao and Yan, 2012) and is important in cellular response to DNA damage (Meas and Mao, 2015). The modification induced by Small Ubiquitin-like Modifier (or SUMO) proteins is called sumoylation of histones. Instead of promoting protein degradation, sumoylation seems to reduce transcriptional activity and to influence the enzymatic activity of histone modifying enzymes, such as histone deacetylase. (Shanmugam et al., 2018; Shiio and Eisenman, 2003). Also sumoylation of histones is involved in regulatory processes in cancer cells. It was observed that interfering with SUMO-1 (one of the three 3 SUMO family

members identified) gene expression could reduce proliferation of endometrial cancer cells and promote apoptosis of endometrial cancer cells by reducing the sumoylation level of histone H4. This finding suggests that SUMO1 could be studied as a new therapeutic target for endometrial carcinoma (Zheng et al., 2015).

Summarizing, PTMs of histones, that include combinations of acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation form part of the "histone code" theory, that refers to chromatin as a dynamic programming platform, which integrates internal and external cellular signal. Since these processes are involved in cancer development, diagnosis and therapies, understanding this histone code may be one of the strategies to diagnose and fight malignancies.

miRNA

The third epigenetic mechanism cited is the expression through the action of specific miRNA. miRNA are short, highly conserved non-coding RNA molecules with gene expression regulatory function. miRNA reduce the expression of target messenger RNA (mRNA) by an effector complex called RNA-induced silencing complex (RISC) resulting in gene silencing by mRNA degradation or translation inhibition (Macfarlane and Murphy, 2010; Poddar et al., 2017). miRNA genes are localized in intergenic or introns regions. During miRNA biogenesis, the miRNA gene is transcribed to form a primary microRNA (pri-miRNA), which undergoes two cleavages to create firstly a precursor microRNA (pre-miRNA) and then a microRNA duplex (miRNA:miRNA). The mature miRNA (contained in the miRNA duplex) may then assemble with RISC. miRNA are involved in the regulation of expression of many oncogenes or tumour suppressor genes (Noorolyai et al., 2018), and their detection is already used in clinical practice to define cancer diagnosis and prognosis (Reddy, 2015). Recently, miRNAs have been also proposed as therapeutic targets for cancer treatment (Mollaei et al., 2019).

In summary, epigenetic mechanisms are essential for gene expression regulation, influencing physiological processes such as differentiation (He et al., 2018), organogenesis (Boland et al., 2014;

Schwanbeck, 2015) and aging (Ashapkin et al., 2017). On the other hand, the deregulation of epigenetic mechanisms is associated with several pathological processes, such as cancer (Fattahi et al., 2018a; Sharma et al., 2010) but also non-neoplastic disorders (e.g cardiovascular diseases, autoimmune diseases, diabetes, and some infectious diseases) (Ogino et al., 2013).

POTENTIAL PHYSIOLOGICAL AND PATOLOGICAL IMPLICATIONS OF EPIGENETIC MODIFICATION INDUCED BY PHYSICAL ACTIVITY

INFLAMMATION, IMMUNE SYSTEM AND CANCER

Inflammatory cytokines and peripheral blood cells

Chronic inflammation is known to play a key role in diseases development and progression. Moderate regular physical activity is related to a decrease of pro-inflammatory cytokines and to an increase of anti-inflammatory cytokines (Cabral-Santos et al., 2018). Several studies investigated if the relation between physical activity and inflammation is mediated by epigenetic mechanisms.

Nakajima et al. explored the epigenetic impact of exercise and age on the methylation of CpG islands in the ASC gene, which is linked with IL-1β and IL-18 secretion and with the initiation of innate immunity. The decrease of methylation of ASC induced by age resulted in an increased pro inflammatory status, but chronic moderate exercise is capable to reduce age-dependent decrease of ASC methylation (Nakajima et al., 2010).

Exercise impacts on inflammation also influencing the expression patterns of miRNAs in leukocytes, such as granulocytes and peripheral blood mononuclear cells. Neutrophils are known to have a key role during acute inflammation, even if evidences indicated that they are also involved in chronic inflammation and adaptive immune responses (Kolaczkowska and Kubes, 2013). Shlomit et al. (Radom-Aizik et al., 2010) focused on the effect of exercise on neutrophil gene expression changes induced by miRNA expression in a group of eleven young, healthy men who performed a series of (ten) 2-min bouts of cycle ergometer exercise alternated with 1-min rest. They found three pathways involved in the inflammation process (Ubiquitin-mediated proteolysis, Jak-STAT

signaling pathway, and Hedgehog signaling pathway) in which miRNA influenced gene expression. The ubiquitin-mediated pathway is known to play a key role in the regulation of immune and inflammatory functions, since it is involved in both canonical and alternative nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways (Iwai, 2014). The Jak-STAT signaling pathway has important immunoregulatory roles: it influences neutrophils, macrophage and lymphocyte functions (O'Shea and Plenge, 2012). The Hedgehog signaling pathway is involved in inflammatory functions such as immune response in tissue damage (Fattahi et al., 2018b; Smelkinson, 2017).

More recently, Shlomit Radom-Aizik et al. (Radom-Aizik et al., 2012) analysed also how miRNAs expression changed with exercise in peripheral blood mononuclear cells (PMNCs). PMNCs refer to lymphocytes (T cells, B cells, NK cells) and monocytes. In this study twelve young men performed brief bouts of heavy exercise and PMNCs were taken before and after exercise. The authors found that exercise altered the expression level of 34 microRNAs, many of which are related to inflammatory processes, such as miR-132[↑], 125b[↓] and let-7e[↓], which are involved in Toll-like receptor 4 (TLR4) signaling. Comparing the microRNA changes to specific genetic pathways they found 12 pathways, including the transforming growth factor beta (TGF-β) and MAP-Kinase signaling, in which these miRNA were involved. Therefore exercise impacts on the expression of miRNAs that influences inflammation and induces neutrophil and peripheral blood mononuclear cells gene expression changes (Ntanasis-Stathopoulos et al., 2013).

NK cells and the tumour microenvironment

Special emphasis needs to be devoted to the epigenetic status of Natural Killer (NK) cells, since they are part of the tumour microenvironment (TME) that also includes fibroblasts, neuroendocrine cells, adipose cells, immune and inflammatory cells, blood and lymphatic vascular networks and extracellular matrix. TME is now considered as a key player in the processes of cancer initiation, progression, and invasion (Najafi et al., 2018).

NK cells recognize molecules of major histocompatibility complex (MHC) class I located on the surface of self-cells through their killer immunoglobulin-like receptors (KIR) and this interaction inhibits their cytotoxic function. Conversely, the down-regulation or the lack of the expression of MHC-I proteins that characterize cells undergoing malignant transformation is the basis of the mechanisms of NK activation and function. Cancer cells often develop mechanisms to evade NK surveillance by altering molecule expression on their surface or by reducing the expression of activating receptor in NK cells (Dahlberg et al., 2015). Improving the NK cell response against cancer is one of the basis of immunotherapies (Dianat-Moghadam et al., 2018; Hofer and Koehl, 2017). Epigenetic is one of the strategy used by cancer cells to induce immune tolerance in NK cells. For example, a study reports that breast cancer stem-like cells elude NK cell cytotoxicity through the expression of miR20a, which mediate the downregulation of MHC class I-related chain A and B (MICA and MICB), two ligands for the NK cell–activating receptor NKG2D (Wang et al., 2014).

Physical activity has several epigenetic consequences on natural killer cells. Radom-Aizik et al. (Radom-Aizik et al., 2013) studied NK cell gene and microRNA (miRNA) expression in thirteen healthy young men who performed ten 2-min bouts of heavy cycle ergometer exercise. NK cells were isolated before and immediately after the exercise. Results showed that a single bout of exercise influences the expression of 986 genes and 23 miRNAs of NK-cells. Intersecting analysis of gene and miRNA expression revealed that some of these miRNA were involved in the regulation of seven pathways related to cancer and cell communication, such as p53 signaling pathway (for example: miRNA hsa-let-7e targets 566 genes, 26 of them are involved in p53 signaling pathway), focal adhesion and adherent junction pathway. This study suggests that exercise directly influences NK cell gene pathways that are involved in cancer processes and surveillance; therefore, we can assume that exercise influences the role of NK cells in tumour suppression.

Zimmer et al. (Zimmer et al., 2014) focused on the epigenetic effect of PA on tumour-competitive lymphocytes. They compared thirty Non-Hodgkin-Lymphoma patients with ten healthy controls,

randomized into two groups: intervention (that exercised once for 30 min at moderate intensity on a bicycle ergometer) and control group. Results evidenced that a single bout of exercise increased histone 4, lysine 5 (H4K5) acetylation in CD8+ T-lymphocytes, suggesting that exercise influences the activity of tumour-competitive lymphocytes.

More recently, the same group (Zimmer et al., 2015) studied the epigenetic modification induced by an intense endurance run (half marathon) on natural killer (NK) cells induced by an intense endurance run (half marathon) in 28 participants (14 cancer patients compared to 14 healthy controls). They found that a single bout of exercise induced a 24-hour-long elevation of histone acetylation and expression of NKG2D gene that encodes a NK-cell receptor activated after the recognition of ligands that are overexpressed on neoplastic cells. Since histone acetylation is associated with enhanced transcriptional activity and since NKG2D can be used as a functional marker of NK activity, these studies confirm that exercise impacts on NK cell activity by epigenetic effects.

Given that epigenetic processes modulate the ability of NK cells to tackle cancer cells, understanding the epigenetic regulation of NK cell function and the mechanisms through which exercise influences this function may be the key to elaborate new cancer prevention strategies and treatment approaches (see Table 1).

Methylation of tumour suppressor genes and repetitive sequences

It is known that aberrant DNA methylation patterns play a significant role in cancer development. In particular the process of carcinogenesis is commonly associated with hypermethylation of tumour-suppressor genes (Zaidi et al., 2013). Studies showed that physical activity modifies the methylation status of tumour suppressor genes.

Coyle et al. (Coyle et al., 2007) studied the methylation status of the promoters of the tumour suppressor genes APC and RASSF1A in 45 healthy women. The hypermethylation of these promoters can be used as an epigenetic marker of breast cancer risk since these genes have been

303 associated with breast cancer development. Results showed that physical exercise reduces or
304 reverses promoter hypermethylation APC and RASSF1A genes in non-malignant breast tissue,
305 allowing their expression.
306 Zeng et al. showed that moderate-intensity aerobic exercise is linked with demethylation of genes
307 whose expression is associated with better breast cancer survival. In particular, they found that
308 exercise lowers L3MBTL1 methylation, causing an increase in its expression. L3MBTL1 is a
309 tumour suppressor gene and an high expression of L3MBTL1 was associated with reduced risk of
310 breast cancer recurrence and mortality (Zeng et al., 2012).
311 Yuasa et al. analysed the relationship between DNA methylation status of tumor-related genes in
312 patient with gastric carcinoma and the patients' lifestyles; they observed that methylation of the
313 CACNA2D3 tumor suppressor gene was inversely correlated with physical activity (Yuasa et al.,
314 2009).
315 About methylation, it is important to remember that methylation determines the silencing of genes
316 and in the case of tumour-suppressor genes causes an increased risk of cancer development,
317 whereas methylation of oncogenes is a mechanism that reduces cancer risk, because it decreases the
318 expression of the oncogene. Thus, it's important to focus on the kind of genes involved by
319 methylation to evaluate the relationship between the hypo/hyper methylation and the global cancer
320 risk.
321 In fact, the alteration of the DNA methylation pattern in cancer not only affects tumour suppressor
322 genes, but also repetitive sequences (e.g. long interspersed nuclear elements, LINEs) that are
323 normally highly methylated and whose hypomethylation results in an increased cancer risk
324 (Grazioli et al., 2017). A study conducted on 161 healthy adult individuals found a connection
325 between global DNA methylation levels measured by detecting LINE-1 sequences and physical
326 activity: subjects who exercised about 30 min/day showed higher global DNA methylation levels
327 compared to those with <10 min/day (Zhang et al., 2011).
328 The effect of physical activity on cancer risk through epigenetic was also studied by Bryan et al.,

(Bryan et al., 2013) who focused on DNA methylation at 45 CpG sites in genes associated with breast cancer. This study involved 64 healthy adults who were randomized into a one-year-long exercise promotion intervention (about 30–50 min of treadmill exercise, 3–5 days per week for 36 weeks). The results showed that participants who exercised more minutes per week had lower levels of DNA methylation, suggesting that higher levels of PA provide a "healthier" methylation profile of CpG islands of genes linked to cancer development.

Therefore, according to these authors, higher levels of physical activity were associated with a "low cancer risk" methylation profile, independently from the methylation level, but depending on the gene(s) or DNA sequences involved (Bryan et al., 2013).

BRAIN TISSUE, NEUROTROPHIC FACTOR AND NEURODEGENERATIVE DISEASE

Epigenetic effect of exercise on BDNF and VEGF

It is known that physical exercise has a positive effect on brain tissue: it increases cognition, improves memory function, enhances neurogenesis and has been promoted as a possible strategy of prevention for neurodegenerative diseases (Meeusen, 2014).

One of the most studied effects of physical activity on brain tissue is the augmented expression of brain-derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophin family found in central and peripheral nervous systems. BDNF is known to play a substantial role in development, plasticity, differentiation, and survival of neurons (Mackay et al., 2017). In particular, it has been demonstrated that BDNF levels increase immediately after a single session of aerobic exercise (Szuhany et al., 2015).

Gomez-Pinilla et al. (Gomez-Pinilla et al., 2011) analyzed the impact of physical exercise on BDNF by mechanisms of epigenetic regulation and found that regular exercise stimulates DNA demethylation of the CpG region of BDNF exon 4 promoter, resulting in increased BDNF mRNA (41%) and protein (30%) in rat hippocampi. This suggests that DNA methylation may be a crucial step by which exercise regulates BDNF expression. In the same study, chromatin

immunoprecipitation assay showed that exercise increases acetylation of histone H3 within the BDNF promoter IV sequence. The specific action of PA on histone H3 could lead to facilitation of BDNF transcription. In addition, they studied the exercise's influence on intracellular signaling that regulates BDNF: in particular, they focused on Ca2+/calmodulin-dependent protein kinase II (CaMKII) and cAMP response element binding protein (CREB). In fact CaMKII activation can lead to phosphorylation of CREB, which recruits CREB-binding protein (CBS) that is known to have a strong histone acetylation transferase-promoting activity, resulting in an increased BDNF transcription. In addition CaMKII and CREB are involved in BDNF-mediated synaptic plasticity and cognition. They found that exercise increased phospho-CREB/CREB ratio by 53% and elevated as well the ratio of phospho-CaMKII when compared to sedentary rats.

Sølvsten et al. (Solvsten et al., 2017) focalized on the expression of Vascular endothelial growth factor A (VEGFA), a signaling factor important for angiogenesis, vasculogenesis, and neurogenesis. They found that exercise determined an augmented expression of VegfA, and they correlated this finding with a reduction of DNA methylation at specific CpG site located within a VegfA promoter Sp1/Sp3 transcription factor recognition element.

Epigenetic effect of physical exercise on DNA methylation and histone modifications

Since the study of epigenetic and gene expression mechanisms of nervous tissue are not acceptable in humans for ethical reasons, most of the studies have been carried out on murine models.

Kashimoto et al. (Kashimoto et al., 2016) evaluated the effects of physical exercise on global DNA methylation in rat brain. They reported that physical exercise increased the global DNA methylation profile of rat's hypothalamus, hippocampus and cortex.

Nevertheless, Sølvsten et al. (Solvsten et al., 2018) found a decreased DNMT3b mRNA expression in the hippocampi of rats that were engaged in physical exercise, suggesting that exercise brings to a specific modulation of methylation in hippocampus.

According to this hypothesis, Abel et al. (Harshman et al., 2014) found that one week of wheel running was associated with a decreased expression pattern of DNMTs (DNMT1, DNMT3A and DNMT3B) in rat hippocampus. A repressed DNMTs gene expression, paired with a highly significant increase in BDNF in the hippocampus of rats in response to exercise, suggest that reduced DNMT expression in response to exercise may be responsible for up-regulated BDNF activity. These findings demonstrate that exercise or sensory stimulation drives the direction of epigenetic and downstream gene changes that occur in the hippocampus.

Findings also suggest a role for aerobic exercise in histone modifications. Elsner et al. (Elsner et al., 2011) studied the effect of exercise on histone HDAC and HAT activities and analyzed the HAT/HDAC ratio that is indicative of histone hyperacetylation status in rat whole hippocampus after treadmill. The single session of treadmill exercise reduced HDAC activity, increased HAT activity and increased the HAT/HDAC balance in rat hippocampus immediately and 1 h after exercise, driving to high transcriptional activity and gene expression.

Moreover, Abel et al. (Abel and Rissman, 2013) observed that one week of wheel running is related to an increase in global acetylation of histone 3 (H3) in the hippocampus and in the cerebellum of young rats. Since increased global H3 acetylation is associated with enhanced gene transcription, and given that H3 acetylation is correlated with increased BDNF in the hippocampus, these findings confirm that physical activity activated epigenetic mechanisms that regulated synaptic plasticity.

They also found that the expression pattern of HDACs decreased in both regions with exercise. These two findings are in line with Elsner's study, strengthening that exercise dependent neuronal effects may be related to acetylation levels through modulation of HAT and HDAC activities. These data support the hypothesis that exercise neuroprotective effects may be related, at least in part, to epigenetics mechanisms, such as global DNA methylation, regulation of growth factor expression and acetylation levels through modulation of HAT and HD.

Epigenetic effect of physical exercise on neurodegenerative disease and spinal cord injury

Experimental and clinical studies suggest that the deregulation of epigenetic mechanisms plays a significant role in neurological diseases. In fact, several studies suggest that epigenetic mechanisms are involved in neurological disorders (like epilepsy and schizophrenia) and neurodegenerative diseases (such as Alzheimer and Parkinson) (Coppede, 2012; Grazioli et al., 2017). An emerging body of evidence recognizes PA as one of the most effective action to improve several aspects of brain-related diseases, such as mood, cognition and sleep in Parkinson disease (Reynolds et al., 2016) and cognitive function in traumatic brain injury and Alzheimer disease (Chin et al., 2015; Intlekofer and Cotman, 2013).

A recent work conducted on subject affected by neurodegenerative diseases found a link between exercise training and levels of global histone H4 acetylation in peripheral blood. Seventeen individuals affected by schizophrenia were encouraged to exercise 1 h, 3 times/week following a program that included aerobic and strength training. Results showed that this exercise protocol induced significant reduction of histone H4 acetylation status in PBMCs, suggesting a decreased transcriptional activity and gene expression. In fact, the reduction of global histone H4 acetylation status in PBMCs results in a lower production of cytokines, such as IL-6, INF-γ and TNF-α, influencing both the natural and acquired immune system. Even if the physiopathology of schizophrenia remains unknown, several studies reported inflammation (sustained by the above-mentioned cytokines) as one of the factor involved in schizophrenia pathogenesis. Although with limitations, this study suggests that exercise induces epigenetic changes in PBMC cells of psychiatric patients, resulting in immune system modulation of patients with schizophrenia (Lavratti et al., 2017).

We therefore could assume that the positive effect of physical exercise on neurodegenerative diseases depends also on epigenetic mechanisms.

Exercise has also been implicated in the rehabilitation of the damaged central nervous system. There is strong, consistent evidence that exercise can improve cardiorespiratory fitness, muscular

strength and reduce depression in people with spinal cord injury (Tweedy et al., 2017). Emerging evidences suggest a role of aerobic exercise mediated by miRNA in spinal cord rehabilitation (Denham et al., 2013; Ganzer et al., 2018). Due to the highly invasive nature of collecting brain tissue in patient with spinal cord injury, rodents have been used for these studies. After a complete spinal cord transection, rats undergo a hind limb exercise (Ex), a passive form of cycling exercise implicated in promoting spinal cord plasticity. Results showed that inflammation and apoptosis associated with spinal cord injury resulted attenuated via reduced spinal cord miR-15b and augmented miR-21 after 5 days of hind limb exercise in rats. miRNA 15 appears to function as a pro-apoptotic factor by reducing the expression of the anti-apoptotic factor Bcl-2 and increasing the expression of caspases 3, 8 and 9 (Liu et al., 2010). Instead, miR-21 works as an anti-apoptotic mediator in spinal cord injury by inhibiting the expression of pro-apoptotic proteins Phosphatase and tensin homolog (PTEN) and programmed cell death protein (PDCD4) (Ning et al., 2014). Therefore, initial exercise may be important to reduce spinal cord injury-associated apoptosis and this probably involve PTEN/mammalian target of rapamycin (mTOR) signalling pathway (Liu et al., 2012).

The emerging results from human and murine studies suggest a role of physical exercise mediated by epigenetic mechanisms in brain neurogenesis, plasticity and damage repair. Thus, up to now it's possible to imagine that exercise intervention may prevent and support treatments of neurodegenerative and traumatic disease (see Table 1).

AGING AND NEURONAL AGING PROCESS

The aging process

Aging is a gradual process that consists in the accumulation of different detrimental changes occurring in cells and tissues that makes the individual more susceptible to environmental challenges and diseases, frailty, or disability. In fact, advancing age is the main risk factor for several chronic diseases in humans (Tosato et al., 2007). Aging process is associated with changes

that involve physical but also environmental, psychological, behavioral, and social processes. Modifications associated with age can be explained by changes in physiological mechanisms, biological processes, molecular pathways and gene expression. There is consistent evidence that epigenetic changes have a huge influence on the aging process (Pal and Tyler, 2016).

Epigenetic changes in aging and the prediction of biological age

The epigenetic modifications determined by aging occur at the various levels described in the introduction of this review: DNA methylation, histone modification and expression of miRNA. The epigenetic mark of aging that has been most extensively studied is DNA methylation. Changes in DNA methylation occur with age and refers both to specific CpG sites and to other regions across the genome (Jones et al., 2015).

In neonatal blood cells, DNA methylation levels are lower than in all the rest of life; the first year is characterized by an increase of median global DNA methylation levels, especially in some regions (CpG island shores and shelves, enhancers, and promoters lacking CpG islands). In fact, DNA methylation is important to silence genes and to regulate expression in developmental stages. After the first year, the average global DNA methylation levels remain stable, with the exception of specific regions codifying for proteins involved in immune pathways that frequently gain DNA methylation (Martino et al., 2011). Curiously, comparing the pattern of DNA methylation of identical twins, studies found that it becomes progressively divergent through years, according to the "epigenetic drift" caused by environmental factors and casual errors (Martino et al., 2013). However, some of the methylation changes that occur with age are directional and repetitive, suggesting that they are associated with biological mechanisms involved in the aging process. In particular, studies showed that almost one-third of the sites reveal age-associated DNA methylation changes, of which about 60% become hypomethylated and 40% hypermethylated upon aging (Florath et al., 2014; Johansson et al., 2013). The DNA methylation sites interested by age dependent hypermethylation are regions rich in CpG islands and DNA methylation sites located

within regions that are involved in transcription regulation (such as promoters). On the other hand age dependent hypomethylation mostly occurs outside CpG islands regions and in repetitive Alu elements (Pal and Tyler, 2016).

According to the reproducibility of DNA methylation changes during aging at some sites, it is possible to predict the biological age of an individual through analyzing his/her DNA methylation pattern. The concept of "biological age", also known as "physiological age", depends on the biological condition of the single individual, considering risk factors such as diet, exercise and sleeping habits. In fact, the aging process can be measured by the traditional chronological age or by concepts related to the biological age referring to the functional capability of a person or organ and its changes with age. The "Epigenetic age" is defined as the estimation age in years resulting from a mathematical algorithm based on the methylation levels of specific CpG islands in the genome (Horvath and Raj, 2018). A recent review of different types of potential biological age methods found that epigenetic age is the most promising molecular estimator (Jylhava et al., 2017).

Positive effect of exercise in the aging process

Aging is associated with several changes that concern various organs and tissues. Exercise is known to attenuate the major hallmarks of aging by acting in a multi-system way. Old people progressively lose cardiorespiratory fitness and muscle mass leading to sarcopenia and resulting in the loss of functional independence and in the development of a frailty syndrome, characterized by weakness, slowness (low walking speed), low level of physical activity, low energy or self-reported exhaustion, and unintentional weight loss (Garatachea et al., 2015).

There is a plethora of studies demonstrating that an active lifestyle and the regular practice of PA improve cardiovascular health and in parallel mitigate the impact of risk factors affecting cardio-metabolic and brain health. Furthermore, PA provides positive effects at cognitive and psychological levels, including prevention and reduction of depressive conditions and anxiety

disorders, stress decrease, enhanced self-confidence and delayed cognitive decline in the elderly (Kaliman et al., 2011; Kokkinos and Myers, 2010; Zanuso et al., 2010).

With a focus at the "cellular level", López-Otín et al. (Lopez-Otin et al., 2013) described nine hallmarks that represent common denominators of aging: genomic instability, telomere attrition, loss of protein homeostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, and epigenetic alterations. Most of these hallmarks are influenced by exercise. For example, Safdar et al. (Safdar et al., 2011) found that a 5-month endurance (aerobic) exercise is able to prevent mitochondrial DNA instability in a murine model of progeroid aging. On the other hand, there are studies supporting a link between usual aerobic exercise, and longer leukocyte telomere length, thus suggesting that exercise modulates telomerase enzyme activity (Denham et al., 2013; Laye et al., 2012).

Protein homeostasis consists in refolding or degrading altered proteins by different processes such as autophagy. Aging is characterized by a progressive damage of these processes, leading to an accumulation and aggregation of proteins associated with neurodegenerative pathologies in elder people (Koga et al., 2011). The autophagy process is strengthen by exercise in multiple organs involved in metabolic regulation, such as muscle, liver, pancreas and adipose tissue, but also in the brain tissue (He et al., 2012).

Exercise also positively influences the metabolism, counteracting the effects of aging: it increases insulin sensitivity (Mann et al., 2014)and promotes protein synthesis in the muscles, preventing sarcopenia (Glover and Phillips, 2010). Regular exercise has a positive impact in the mitochondrial function by several mechanisms such as the increase of levels of mitochondrial proteins expression (Rebelo-Marques et al., 2018).

Aging and age-related pathologies are characterized by cellular senescence, which refers to a stable slowing down until arrest of the cell cycle associated with stereotyped phenotypic modifications (Kuilman et al., 2010). This process is in part regulated by telomere-associated proteins (directly influenced by exercise) but is also stimulated by non-telomeric DNA damage.

Stem cell exhaustion consists in the progressive number and functionality reduction of stem cells in all tissues, but especially in the myogenic one, where stem cells are known as satellite cells. Exercise promotes the proliferation of different adult stem cell (such as mesenchymal and hematopoietic stem cells) and counteracts the age-associated reduction in reparative capacity of endothelial progenitor cells (Fiuza-Luces et al., 2014; Xia et al., 2012).

In addition, also intercellular communication is affected by aging, due to an increased inflammatory status associated with progressive aging, named "inflammaging", that is attenuated by exercise, thanks to its anti-inflammatory potential (Abd El-Kader and Al-Shreef, 2018; Salminen et al., 2012)

Epigenetic mechanisms on the basis of positive effect of exercise in aging process

Exercise appears capable to induce epigenetic modifications that could attenuate age effect.

One of the most studied epigenetic effects of exercise concerns the age-related nervous tissue change. We have already explained that PA exerts neuroprotective effects by inducing changes in the transcriptional profiles of growth and neurotrophic factors such as VEGF and BDNF that promotes neurogenesis and neuroplasticity in the brain. The above-mentioned studies analysed the epigenetic effect of exercise in the hippocampus of murine models. Interestingly, the neurotrophic effect induced by exercise seems to change according to age. One week of wheel running exercise increased BDNF protein levels in both young (2 months), middle aged (15 months) and old (24 months) rodents, but only the young group maintained a significant increase in this factor above basal levels after 4 weeks of exposure (Adlard et al., 2005). This result shows that the effect of exercise on BDNF expression is the same at all ages, but exercise has a more long-lasting impact on young animals, suggesting that a long-term physical activity should be planned to obtain a lasting effect on BDNF in the elderly group.

Elsner et al. (Elsner et al., 2013) focused on the epigenetic effect of exercise at different ages, comparing the effect of exercise on histone H3 at lysine 9 (H3-K9) methylation levels in hippocampus of young and adult Wistar rats (specifically 3 and 20-months old). After showing

563 lower H3-K9 methylation (i.e. transcriptional activation) levels in 20-months-old rats hippocampi
564 compared to the young group, the animals were submitted to two different exercise protocols: a
565 single session of running or a two-week treadmill protocol. They found that both exercise protocols
566 reduced H3-K9 methylation levels in young rats. On the contrary, in the aged group the single
567 session induced higher H3-K9 methylation levels, whereas the chronic protocol didn't modify H3-
568 K9 methylation levels. Thus, exercise had an opposite effect on H3-K9 methylation levels when
569 comparing young adult and old groups. Results suggest that the single session reversed the changes
570 on H3-K9 methylation levels induced by aging. Therefore, histone lysines might be methylated in
571 response to exercise with different patterns (mono-, di- or tri- methylation) depending on the age
572 (Elsner et al., 2013).
573 The same group measured hippocampus pro and anti-inflammatory cytokines levels, hippocampus
574 histone H4 acetylation levels and evaluated aversive memory through inhibitory avoidance task.
575 Rats of 3 and 20 months of age were assigned to non-exercised (sedentary) and exercised (running
576 daily for 20 min for 2 weeks) groups. They found that the exercise protocol ameliorated aging-
577 related memory decline, reduced pro-inflammatory markers and increased histone H4 acetylation
578 levels in hippocampi of 20- months-old rats, and increased IL-4 (an anti-inflammatory cytokine)
579 levels in hippocampi of both groups, but more acutely in young adults rats. Global H4
580 hyperacetylation in hippocampi of exercised aged rats results positively correlated with the
581 inhibitory avoidance aversive memory performance. These results found a relationship between
582 age-related aversive memory impairment, the imbalance of inflammatory and PA and epigenetic
583 parameters (Lovatel et al., 2013).
584 More recently, Cechinel et al. (Cechinel et al., 2016) investigated the effect of treadmill exercise on
585 H4 acetylation in prefrontal cortices from 3 and 21-months aged rats. They found that a treadmill
586 protocol of 20 min/day during 14 days induced an increase in histone H4 acetylation levels in
587 prefrontal cortices of 21-months-old rats, with no changes in young group. This study demonstrated
588 that moderate daily exercise induces cortical acetylation in aged rats, suggesting that prefrontal

cortex from aged rats is more susceptible to exercise than young adult ones. Taken together, these results suggest that epigenetic modifications both in hippocampus and in prefrontal cortex are influenced in an age-dependent way by physical exercise.

Daniele et al. (Daniele et al., 2018) explored the effects of physical exercise on epigenetic regulation of α-synuclein (SNCA, the misfolded protein that accumulate in in Lewy bodies that characterized Parkinson's disease) in blood samples of ageing healthy subject. More specifically, they compared blood intron1-SNCA ($SNCA_{I1}$) CpG islands methylation status of young and older endurance athletes and healthy sedentary controls. Results showed that the $SNCA_{I1}$ methylation status increased with ageing, and consistently with this result, low α-synuclein levels were found in the blood of aged subjects. This study report that SNCA methylation levels directly correlate with age and physical exercise induced changes on the SNCA methylation status and consequently protein levels of α-synuclein, suggesting a possible role of exercise in preventing proteine accumulation. Summarizing, we can assume that epigenetic regulation benefits from the effects of physical activity against age-related neurodegeneration.

CONCLUSIONS

Trying to answer the questions that we postulated in the introduction of this review, we can draw some conclusions.

First, there are evidences that report that PA has a particular influence on epigenetic mechanisms, both in inflammation and in cancer, neurodegenerative diseases and aging process (see table 1).

Second, there is a role of PA and epigenetic mechanisms in the prevention of several diseases: higher levels of physical activity are associated with a lower cancer risk methylation profile, confirming that PA plays a key role in the prevention of cancer. In addition, exercise is an effective strategy of prevention for neurodegenerative diseases (such as Parkinson disease) and for contrasting neuronal aging because it influences the epigenetic regulation of neurotrophic factor and it modulates epigenetic processes in regions crucial for memory processes (such as hippocampus).

Third, this influence can be used for several purposes: considering the role of epigenetic modulation induced by PA on NK cells and tumour microenvironment, is possible that the advancement of research in this modulation may provide new cancer prevention and treatment approaches . Moreover, the DNA methylation patterns of specific CpG islands in the genome can be used to estimate the "Epigenetic age", suggesting that epigenetic analysis may assume a diagnostic as well as a predictive significance.

Thus, by modulating epigenetic processes, PA influences prevention, development and the course of inflammatory and cancer diseases, as well as neurodegenerative illnesses.

AUTHOR CONTRIBUTION

M.F., G.Z. and L.M.N. conceived and designed the review; M.F. and L.M.N. wrote the manuscript; P.M., D.M. and P.M. analyzed the data of the manuscript; all authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS AND GRANT SUPPORT

This work was supported by current University of Ferrara research funds FAR, FIR (G.Z. and L.M.N.) and by LILT – Sezione Provinciale di Ferrara (L.M.N.).

REFERENCES

Abd, El-Kader S.M., Al-Shreef, F.M. (2018). Inflammatory cytokines and immune system modulation by aerobic versus resisted exercise training for elderly. *Afr Health Sci*, 18,120-131.

Abel, J.L., Rissman, E.F. (2013). Running-induced epigenetic and gene expression changes in the adolescent brain. *Int J Dev Neurosci*, 31, 382-390.

Adlard, P.A., Perreau, V.M., Cotman, C.W. (2005). The exercise-induced expression of BDNF within the hippocampus varies across life-span. *Neurobiol Aging*, 26, 511-520.

Ashapkin, V.V., Kutueva, L.I., Vanyushin, B.F. (2017). Aging as an Epigenetic Phenomenon. *Curr Genomics*, 18, 385-407.

Barone, D., Cito, L., Tommonaro, G., Abate, A.A., Penon, D., De Prisco, R., Penon, A., Forte, I.M., Benedetti, E., Cimini, A., Indovina, P., Nicolaus, B., Pentimalli, F., Giordano, A. (2018). Antitumoral potential, antioxidant activity and carotenoid content of two Southern Italy tomato cultivars extracts: San Marzano and Corbarino. *J Cell Physiol*, 233, 1266-1277.

Boland, M.J., Nazor, K.L., Loring, J.F. (2014). Epigenetic regulation of pluripotency and differentiation. *Circ Res*, 115, 311-324.

Brehove, M., Wang, T., North, J., Luo, Y., Dreher, S.J., Shimko, J.C., Ottesen, J.J., Luger, K., Poirier, M.G. (2015). Histone core phosphorylation regulates DNA accessibility. *J Biol Chem*, 290, 22612-22621.

Bryan, A.D., Magnan, R.E., Hooper, A.E., Harlaar, N., Hutchison, K.E. (2013). Physical activity and differential methylation of breast cancer genes assayed from saliva: a preliminary investigation. *Ann Behav Med*, 45, 89-98.

Cabral-Santos, C., de Lima Junior, E.A., Fernandes, I., Pinto, R.Z., Rosa-Neto, J.C., Bishop, N.C., Lira, F.S. (2018). Interleukin-10 responses from acute exercise in healthy subjects: A systematic review. *J Cell Physiol*. doi: 10.1002/jcp.27920.

Cao, J., Yan, Q. (2012). Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front Oncol*, 2:26.

Cechinel, L.R., Basso, C.G., Bertoldi, K., Schallenberger, B., de Meireles, L.C., Siqueira, I.R. (2016). Treadmill exercise induces age and protocol-dependent epigenetic changes in prefrontal cortex of Wistar rats. *Behav Brain Res*, 313, 82-87.

Chi, P., Allis, C.D., Wang, G.G. (2010). Covalent histone modifications--miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer*, 10, 457-469.

Chin, L.M., Keyser, R.E., Dsurney, J., Chan, L. (2015). Improved cognitive performance following aerobic exercise training in people with traumatic brain injury. *Arch Phys Med Rehabil*, 96, 754-759.

Condello, G., Ling, F.C., Bianco, A., Chastin, S., Cardon, G., Ciarapica, D., Conte, D., Cortis, C., De Craemer, M., Di Blasio, A., Gjaka, M., Hansen, S., Holdsworth, M., Iacoviello, L., Izzicupo, P., Jaeschke, L., Leone, L., Manoni, L., Menescardi, C., Migliaccio, S., Nazare, J.A., Perchoux, C., Pesce, C., Pierik, F., Pischon, T., Polito, A., Puggina, A., Sannella, A., Schlicht, W., Schulz, H., Simon, C., Steinbrecher, A., MacDonncha, C., Capranica, L.,

consortium D. (2016). Using concept mapping in the development of the EU-PAD framework (EUropean-Physical Activity Determinants across the life course): a DEDIPAC-study. *BMC Public Health*, 16, 1145.

Coppede, F. (2012). Genetics and epigenetics of Parkinson's disease. *ScientificWorldJournal*, 2012, 489830.

Coyle, Y.M., Xie, X.J., Lewis, C.M., Bu, D., Milchgrub, S., Euhus, D.M. (2007). Role of physical activity in modulating breast cancer risk as defined by APC and RASSF1A promoter hypermethylation in nonmalignant breast tissue. *Cancer Epidemiol Biomarkers Prev*, 6, 192-196.

Dahlberg, C.I., Sarhan, D., Chrobok, M., Duru, A.D., Alici, E. (2015). Natural Killer Cell-Based Therapies Targeting Cancer: Possible Strategies to Gain and Sustain Anti-Tumor Activity. *Front Immunol*, 6, 605.

Daniele, S., Costa, B., Pietrobono, D., Giacomelli, C., Iofrida, C., Trincavelli, M.L., Fusi, J., Franzoni, F., Martini, C. (2018). Epigenetic Modifications of the alpha-Synuclein Gene and Relative Protein Content Are Affected by Ageing and Physical Exercise in Blood from Healthy Subjects. *Oxid Med Cell Longev*, 2018, 3740345.

Daskalaki, M.G., Tsatsanis, C., Kampranis, S.C. (2018). Histone methylation and acetylation in macrophages as a mechanism for regulation of inflammatory responses. *J Cell Physiol*, 233. 6495-6507.

Denham, J., Nelson, C.P., O'Brien, B.J., Nankervis, S.A., Denniff, M., Harvey, J.T., Marques, F.Z., Codd, V., Zukowska-Szczechowska, E., Samani, N.J., Tomaszewski, M., Charchar, F.J. (2013). Longer leukocyte telomeres are associated with ultra-endurance exercise independent of cardiovascular risk factors. *PLoS One*, 8, e69377.

Dianat-Moghadam, H., Rokni, M., Marofi, F., Panahi, Y., Yousefi, M. (2018). Natural killer cell-based immunotherapy: From transplantation toward targeting cancer stem cells. *J Cell Physiol*, 234, 259-273.

Donohoe, D.R., Bultman, S.J. (2012). Metaboloepigenetics: interrelationships between energy metabolism and epigenetic control of gene expression. *J Cell Physiol*, 227, 3169-3177.

Eberharter, A., Becker, P.B. (2002). Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. *EMBO Rep*, 3, 224-229.

Ellis, L., Atadja, P.W., Johnstone, R.W. (2009). Epigenetics in cancer: targeting chromatin modifications. *Mol Cancer Ther*, 8, 1409-1420.

712 Elsner, V.R., Lovatel, G.A., Bertoldi, K., Vanzella, C., Santos, F.M., Spindler, C., de Almeida,
713 E.F., Nardin, P., Siqueira, I.R. (2011). Effect of different exercise protocols on histone
714 acetyltransferases and histone deacetylases activities in rat hippocampus. *Neuroscience*,
715 192, 580-587.
716 Elsner, V.R., Lovatel, G.A., Moyses, F., Bertoldi, K., Spindler, C., Cechinel, L.R., Muotri, A.R.,
717 Siqueira, I.R. (2013). Exercise induces age-dependent changes on epigenetic parameters in
718 rat hippocampus: a preliminary study. *Exp Gerontol*, 48, 136-139.
719 Fattahi, S., Kosari-Monfared, M., Ghadami, E., Golpour, M., Khodadadi, P., Ghasemiyan, M.,
720 Akhavan-Niaki, H. (2018a). Infection-associated epigenetic alterations in gastric cancer:
721 New insight in cancer therapy. *J Cell Physiol*, 233, 9261-9270.
722 Fattahi, S,, Pilehchian Langroudi, M., Akhavan-Niaki, H. (2018b). Hedgehog signaling pathway:
723 Epigenetic regulation and role in disease and cancer development. *J Cell Physiol*, 233,
724 5726-5735.
725 Ferioli, M., Zauli, G., Martelli, A.M., Vitale, M., McCubrey, J.A., Ultimo, S., Capitani, S., Neri,
726 L.M. (2018). Impact of physical exercise in cancer survivors during and after antineoplastic
727 treatments. *Oncotarget*, 9, 14005-14034.
728 Fiuza-Luces, C., Delmiro, A., Soares-Miranda, L., Gonzalez-Murillo, A., Martinez-Palacios, J.,
729 Ramirez, M., Lucia, A., Moran, M. (2014). Exercise training can induce cardiac autophagy
730 at end-stage chronic conditions: insights from a graft-versus-host-disease mouse model.
731 *Brain Behav Immun*, 39, 56-60.
732 Florath, I., Butterbach, K., Muller, H., Bewerunge-Hudler, M., Brenner, H. (2014). Cross-sectional
733 and longitudinal changes in DNA methylation with age: an epigenome-wide analysis
734 revealing over 60 novel age-associated CpG sites. *Hum Mol Genet*, 23, 1186-1201.
735 Ganzer, P.D., Beringer, C.R., Shumsky, J.S., Nwaobasi, C., Moxon, K.A. (2018). Serotonin
736 receptor and dendritic plasticity in the spinal cord mediated by chronic serotonergic
737 pharmacotherapy combined with exercise following complete SCI in the adult rat. *Exp*
738 *Neurol*, 304, 132-142.
739 Garatachea, N., Pareja-Galeano, H., Sanchis-Gomar, F., Santos-Lozano, A., Fiuza-Luces, C.,
740 Moran, M., Emanuele, E., Joyner, M.J., Lucia, A. (2015). Exercise attenuates the major
741 hallmarks of aging. *Rejuvenation Res*, 18, 57-89.
742 Ghosh, A.K., Rai, R., Flevaris, P., Vaughan, D.E. (2017). Epigenetics in Reactive and Reparative
743 Cardiac Fibrogenesis: The Promise of Epigenetic Therapy. *J Cell Physiol*, 232, 1941-1956.

744 Glover, E.I., Phillips, S.M. (2010). Resistance exercise and appropriate nutrition to counteract
745 muscle wasting and promote muscle hypertrophy. *Curr Opin Clin Nutr Metab Care*, 13,
746 630-634.
747 Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z., Fan, G. (2011). Exercise impacts brain-derived
748 neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. *Eur J*
749 *Neurosci*, 33, 383-390.
750 Grazioli, E., Dimauro, I., Mercatelli, N., Wang, G., Pitsiladis, Y., Di Luigi, L., Caporossi, D.
751 (2017). Physical activity in the prevention of human diseases: role of epigenetic
752 modifications. *BMC Genomics*, 18, 802.
753 Greer, E.L., Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance.
754 *Nat Rev Genet*, 13, 343-357.
755 Harshman, S.W., Hoover, M.E., Huang, C., Branson, O.E., Chaney, S.B., Cheney, C.M., Rosol,
756 T.J., Shapiro, C.L., Wysocki, V.H., Huebner, K., Freitas, M.A. (2014). Histone H1
757 phosphorylation in breast cancer. *J Proteome Res*, 13, 2453-2467.
758 He, C., Sumpter, R.Jr., Levine, B. (2012). Exercise induces autophagy in peripheral tissues and in
759 the brain. *Autophagy*, 8, 1548-1551.
760 He, Y., Ling, S., Sun, Y., Sheng, Z., Chen, Z., Pan, X., Ma, G. (2018). DNA methylation regulates
761 alpha-smooth muscle actin expression during cardiac fibroblast differentiation. *J Cell*
762 *Physiol*, doi: 10.1002/jcp.27471
763 Hofer, E., Koehl, U. (2017). Natural Killer Cell-Based Cancer Immunotherapies: From Immune
764 Evasion to Promising Targeted Cellular Therapies. *Front Immunol*, 8, 745.
765 Horvath, S., Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of
766 ageing. *Nat Rev Genet*, 19, 371-384.
767 Intlekofer, K.A., Cotman, C.W. (2013). Exercise counteracts declining hippocampal function in
768 aging and Alzheimer's disease. *Neurobiol Dis*, 57, 47-55.
769 Iwai, K. (2014). Diverse roles of the ubiquitin system in NF-kappaB activation. *Biochim Biophys*
770 *Acta*,1843, 129-136.
771 Jahan, S., Sun, J.M., He, S., Davie, J.R. (2018). Transcription-dependent association of HDAC2
772 with active chromatin. *J Cell Physiol* , 233, 1650-1657.
773 Jang, H.S., Shin, W.J., Lee, J.E., Do, J.T. (2017). CpG and Non-CpG Methylation in Epigenetic
774 Gene Regulation and Brain Function. *Genes (Basel)*, 8.
775 Johansson, A., Enroth, S., Gyllensten, U. (2013). Continuous Aging of the Human DNA
776 Methylome Throughout the Human Lifespan. *PLoS One*, 8, e67378.

Jones, M.J., Goodman, S.J., Kobor, M.S. (2015). DNA methylation and healthy human aging. *Aging Cell*, 14, 924-932.

Jylhava, J., Pedersen, N.L., Hagg, S. (2017). Biological Age Predictors. *EBioMedicine*, 21, 29-36.

Kaliman, P., Parrizas, M., Lalanza, J.F., Camins, A., Escorihuela, R.M., Pallas, M. (2011). Neurophysiological and epigenetic effects of physical exercise on the aging process. *Ageing Res Rev*, 10, 475-486.

Kashimoto, R.K., Toffoli, L.V., Manfredo, M.H.F., Volpini, V.L., Martins-Pinge, M.C., Pelosi, G.G., Gomes, M.V. (2016). Physical exercise affects the epigenetic programming of rat brain and modulates the adaptive response evoked by repeated restraint stress. *Behav Brain Res*, 296, 286-289.

Kim, J.K., Samaranayake, M., Pradhan, S. (2009). Epigenetic mechanisms in mammals. *Cell Mol Life Sci*, 66, 596-612.

Koga, H., Kaushik, S., Cuervo, A.M. (2011). Protein homeostasis and aging: The importance of exquisite quality control. *Ageing Res Rev*, 10, 205-215.

Kohli, R.M., Zhang, Y.. (2013). TET enzymes, TDG and the dynamics of DNA demethylation. *Nature*, 502, 472-479.

Kokkinos, P., Myers, J. (2010). Exercise and physical activity: clinical outcomes and applications. *Circulation*,122, 1637-1648.

Kolaczkowska, E., Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*, 13, 159-175.

Kuilman, T., Michaloglou, C., Mooi, W.J., Peeper, D.S. (2010). The essence of senescence. *Genes Dev*, 24, 2463-2479.

Kurdistani, S.K., Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*, 4, 276-284.

Laurent, L., Wong, E., Li, G., Huynh, T., Tsirigos, A., Ong, C.T., Low, H.M., Kin Sung, K.W., Rigoutsos, I., Loring, J., Wei, C.L. (2010). Dynamic changes in the human methylome during differentiation. *Genome Res*, 20, 320-331.

Lavratti, C., Dorneles, G., Pochmann, D., Peres, A., Bard, A., de Lima Schipper, L., Dal Lago, P., Wagner, L.C., Elsner, V.R. (2017). Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia. *Physiol Behav*, 168, 84-90.

Laye, M.J., Solomon, T.P., Karstoft, K., Pedersen, K.K., Nielsen, S.D., Pedersen, B.K. (2012). Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol (1985)*, 112, 773-781.

Li, G.X., Ding, Z.Y., Wang, Y.W., Liu, T.T., Chen, W.X., Wu, J.J., Xu, W.Q., Zhu, P., Zhang, B.X. (2018). Integrative analysis of DNA methylation and gene expression identify a six epigenetic driver signature for predicting prognosis in hepatocellular carcinoma. *J Cell Physiol*. doi: 10.1002/jcp.27882.

Liu, G., Detloff, M.R., Miller, K.N., Santi, L., Houle, J.D. (2012). Exercise modulates microRNAs that affect the PTEN/mTOR pathway in rats after spinal cord injury. *Exp Neurol*, 233, 447-456.

Liu, G., Keeler, B.E., Zhukareva, V., Houle, J.D. (2010). Cycling exercise affects the expression of apoptosis-associated microRNAs after spinal cord injury in rats. *Exp Neurol*, 226, 200-206.

Liu, N., Li, S., Wu, N., Cho, K.S. (2017). Acetylation and deacetylation in cancer stem-like cells. *Oncotarget*, 8, 89315-89325.

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G. (2013). The hallmarks of aging. *Cell*, 153, 1194-1217.

Lovatel, G.A., Elsner, V.R., Bertoldi, K., Vanzella, C., Moyses Fdos, S., Vizuete, A., Spindler, C., Cechinel, L.R., Netto, C.A., Muotri, A.R., Siqueira, I.R. (2013). Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus. *Neurobiol Learn Mem*, 101, 94-102.

Macfarlane, L.A., Murphy, P.R. (2010). MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics*, 11, 537-561.

Mackay, C.P., Kuys, S.S., Brauer, S.G. (2017). The Effect of Aerobic Exercise on Brain-Derived Neurotrophic Factor in People with Neurological Disorders: A Systematic Review and Meta-Analysis. *Neural Plast*, 2017, 4716197.

Mann, D.A. (2014). Epigenetics in liver disease. *Hepatology*, 60, 1418-1425.

Mann, S., Beedie, C., Balducci, S., Zanuso, S., Allgrove, J., Bertiato, F., Jimenez, A. (2014). Changes in insulin sensitivity in response to different modalities of exercise: a review of the evidence. *Diabetes Metab Res Rev*, 30, 257-268.

Martino, D., Loke, Y.J., Gordon, L., Ollikainen, M., Cruickshank, M.N., Saffery, R., Craig, J.M. (2013). Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. *Genome Biol*, 14, R42.

Martino, D.J., Tulic, M.K., Gordon, L., Hodder, M., Richman, T.R., Metcalfe, J., Prescott, S.L., Saffery, R. (2011). Evidence for age-related and individual-specific changes in DNA methylation profile of mononuclear cells during early immune development in humans. *Epigenetics*, 6, 1085-1094.

McCauley, B.S., Dang, W. (2014). Histone methylation and aging: lessons learned from model systems. *Biochim Biophys Acta*, 1839, 1454-1462.

McGrath, J., Trojer, P. (2015). Targeting histone lysine methylation in cancer. *Pharmacol Ther*, 150, 1-22.

Meas, R., Mao, P.. (2015). Histone ubiquitylation and its roles in transcription and DNA damage response. *DNA Repair*, 36, 36-42.

Meeusen, R. (2014). Exercise, nutrition and the brain. *Sports Med*, 44 Suppl 1, S47-56.

Mollaei, H., Safaralizadeh, R., Rostami, Z. (2019). MicroRNA replacement therapy in cancer. *J Cell Physiol*. doi: 10.1002/jcp.28058.

Najafi, M., Goradel, N.H., Farhood, B., Salehi, E., Solhjoo, S., Toolee, H., Kharazinejad, E., Mortezaee, K. (2018). Tumor microenvironment: Interactions and therapy. *J Cell Physiol*. doi: 10.1002/jcp.27425.

Nakajima, K., Takeoka, M., Mori, M., Hashimoto, S., Sakurai, A., Nose, H., Higuchi, K., Itano, N., Shiohara, M., Oh, T., Taniguchi, S. (2010). Exercise effects on methylation of ASC gene. *Int J Sports Med*, 31, 671-675.

Ning, B., Gao, L., Liu, R.H., Liu, Y., Zhang, N.S., Chen, Z.Y. (2014). microRNAs in spinal cord injury: potential roles and therapeutic implications. *Int J Biol Sci*, 10, 997-1006.

Noorolyai, S., Mokhtarzadeh, A., Baghbani, E., Asadi, M., Baghbanzadeh Kojabad, A., Mogaddam, M.M., Baradaran, B. (2018). The role of microRNAs involved in PI3-kinase signaling pathway in colorectal cancer. *J Cell Physiol*. doi: 10.1002/jcp.27415.

Ntanasis-Stathopoulos, J., Tzanninis, J.G., Philippou, A., Koutsilieris, M. (2013). Epigenetic regulation on gene expression induced by physical exercise. *J Musculoskelet Neuronal Interact*, 13, 133-146.

O'Shea, J.J., Plenge, R. (2012). JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity*, 36, 542-550.

Ogino, S., Lochhead, P., Chan, A.T., Nishihara, R., Cho, E., Wolpin, B.M., Meyerhardt, J.A., Meissner, A., Schernhammer, E.S., Fuchs, C.S., Giovannucci, E. (2013). Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol*, 26, 465-484.

Pal, S., Tyler, J.K. (2016). Epigenetics and aging. *Sci Adv*, 2, e1600584.

Poddar, S., Kesharwani, D., Datta, M. (2017). Interplay between the miRNome and the epigenetic machinery: Implications in health and disease. *J Cell Physiol*, 232, 2938-2945.

Radom-Aizik, S., Zaldivar, F., Haddad, F., Cooper, D.M. (2013). Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. *J Appl Physiol (1985)*, 114, 628-636.

Radom-Aizik, S., Zaldivar, F.Jr., Leu, S.Y., Adams, G.R., Oliver, S., Cooper, D.M. (2012). Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. *Clin Transl Sci*, 5, 32-38.

Radom-Aizik, S., Zaldivar, F.Jr., Oliver, S., Galassetti, P., Cooper, D.M. (2010). Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. *J Appl Physiol (1985)*, 109, 252-261.

Rebelo-Marques, A., De Sousa Lages, A., Andrade, R., Ribeiro, C.F., Mota-Pinto, A., Carrilho, F., Espregueira-Mendes, J. (2018). Aging Hallmarks: The Benefits of Physical Exercise. *Front Endocrinol (Lausanne)*, 9, 258.

Reddy, K.B. (2015). MicroRNA (miRNA) in cancer. *Cancer Cell Int*, 15, 38.

Reynolds, G.O., Otto, M.W., Ellis, T.D., Cronin-Golomb, A. (2016). The Therapeutic Potential of Exercise to Improve Mood, Cognition, and Sleep in Parkinson's Disease. *Mov Disord*, 31, 23-38.

Roostaee, A., Benoit, Y.D., Boudjadi, S., Beaulieu, J.F. (2016). Epigenetics in Intestinal Epithelial Cell Renewal. *J Cell Physiol* , 231, 2361-2367.

Rossetto, D., Avvakumov, N., Cote, J. (2012). Histone phosphorylation: a chromatin modification involved in diverse nuclear events. *Epigenetics*, 7, 1098-1108.

Rothbart, S.B., Strahl, B.D. (2014). Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta*, 1839, 627-643.

Safdar, A., Bourgeois, J.M., Ogborn, D.I., Little, J.P., Hettinga, B.P., Akhtar, M., Thompson, J.E., Melov, S., Mocellin, N.J., Kujoth, G.C., Prolla, T.A., Tarnopolsky, M.A. (2011). Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proc Natl Acad Sci U S A*, 108, 4135-4140.

Salminen, A., Kaarniranta, K., Kauppinen, A. (2012). Inflammaging: disturbed interplay between autophagy and inflammasomes. *Aging (Albany NY)*, 4, 166-175.

Scaffidi, P. (2016). Histone H1 alterations in cancer. *Biochim Biophys Acta*, 1859, 533-539.

Schwanbeck, R. (2015). The role of epigenetic mechanisms in Notch signaling during development. *J Cell Physiol*, 230, 969-981.

Shafabakhsh, R., Aghadavod, E., Ghayour-Mobarhan, M., Ferns, G., Asemi, Z. (2018). Role of histone modification and DNA methylation in signaling pathways involved in diabetic retinopathy. *J Cell Physiol*. doi: 10.1002/jcp.27844.

910 Shanmugam, M.K., Arfuso, F., Arumugam, S., Chinnathambi, A., Jinsong, B., Warrier, S., Wang,
911 L.Z., Kumar, A.P., Ahn, K.S., Sethi, G., Lakshmanan, M. (2018). Role of novel histone
912 modifications in cancer. *Oncotarget*, 9, 11414-11426.
913 Sharma, S., Kelly, T.K., Jones, P.A. (2010). Epigenetics in cancer. *Carcinogenesis*, 31, 27-36.
914 Shiio, Y., Eisenman, R.N. (2003). Histone sumoylation is associated with transcriptional repression.
915 *Proc Natl Acad Sci U S A*, 100, 13225-13230.
916 Sigalotti, L., Fratta, E., Coral, S., Cortini, E., Covre, A., Nicolay, H.J., Anzalone, L., Pezzani, L., Di
917 Giacomo, A.M., Fonsatti, E., Colizzi, F., Altomonte, M., Calabro, L., Maio, M. (2007).
918 Epigenetic drugs as pleiotropic agents in cancer treatment: biomolecular aspects and clinical
919 applications. *J Cell Physiol*, 212, 330-344.
920 Smelkinson, M.G. (2017). The Hedgehog Signaling Pathway Emerges as a Pathogenic Target. *J*
921 *Dev Biol* 5.
922 Solvsten, C.A.E., Daugaard, T.F., Luo, Y., de Paoli, F., Christensen, J.H., Nielsen, A.L. (2017). The
923 Effects of Voluntary Physical Exercise-Activated Neurotrophic Signaling in Rat
924 Hippocampus on mRNA Levels of Downstream Signaling Molecules. *J Mol Neurosci*, 62,
925 142-153.
926 Solvsten, C.A.E., de Paoli, F., Christensen, J.H., Nielsen, A.L. (2018). Voluntary Physical Exercise
927 Induces Expression and Epigenetic Remodeling of VegfA in the Rat Hippocampus. *Mol*
928 *Neurobiol*, 55, 567-582.
929 Szuhany, K.L., Bugatti, M., Otto, M.W. (2015). A meta-analytic review of the effects of exercise on
930 brain-derived neurotrophic factor. *J Psychiatr Res*, 60, 56-64.
931 Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer,
932 L.M., Liu, D.R., Aravind, L., Rao, A. (2009). Conversion of 5-methylcytosine to 5-
933 hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*, 324, 930-935.
934 Telu, K.H., Abbaoui, B., Thomas-Ahner, J.M., Zynger, D.L., Clinton, S.K., Freitas, M.A.,
935 Mortazavi, A. (2013). Alterations of histone H1 phosphorylation during bladder
936 carcinogenesis. *J Proteome Res*, 12, 3317-3326.
937 Tosato, M., Zamboni, V., Ferrini, A., Cesari, M. (2007). The aging process and potential
938 interventions to extend life expectancy. *Clin Interv Aging*, 2, 401-412.
939 Tweedy, S.M., Beckman, E.M., Geraghty, T.J., Theisen, D., Perret, C., Harvey, L.A.,
940 Vanlandewijck, Y.C. (2017). Exercise and sports science Australia (ESSA) position
941 statement on exercise and spinal cord injury. *J Sci Med Sport*, 20, 108-115.
942 Vecera, J., Bartova, E., Krejci, J., Legartova, S., Komurkova, D., Ruda-Kucerova, J., Stark, T.,
943 Drazanova, E., Kasparek, T., Sulcova, A., Dekker, F.J., Szymanski, W., Seiser, C., Weitzer,

G., Mechoulam, R., Micale, V., Kozubek, S. (2018). HDAC1 and HDAC3 underlie dynamic H3K9 acetylation during embryonic neurogenesis and in schizophrenia-like animals. *J Cell Physiol*, 233, 530-548.

Wang, B., Wang, Q., Wang, Z., Jiang, J., Yu, S.C., Ping, Y.F., Yang, J., Xu, S.L., Ye, X.Z., Xu, C., Yang, L., Qian, C., Wang, J.M., Cui, Y.H., Zhang, X., Bian, X.W. (2014). Metastatic consequences of immune escape from NK cell cytotoxicity by human breast cancer stem cells. *Cancer Res*, 74, 5746-5757.

Xia, W.H., Li, J., Su, C., Yang, Z., Chen, L., Wu, F., Zhang, Y.Y., Yu, B.B., Qiu, Y.X., Wang, S.M., Tao, J. (2012). Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. *Aging Cell*, 11, 111-119.

Yuasa, Y., Nagasaki, H., Akiyama, Y., Hashimoto, Y., Takizawa, T., Kojima, K., Kawano, T., Sugihara, K., Imai, K., Nakachi, K. (2009). DNA methylation status is inversely correlated with green tea intake and physical activity in gastric cancer patients. *Int J Cancer*, 124, 2677-2682.

Zaidi, S.K., Van Wijnen, A.J., Lian, J.B., Stein, J.L., Stein, G.S. (2013). Targeting deregulated epigenetic control in cancer. *J Cell Physiol*, 228, 2103-2108.

Zanuso, S., Jimenez, A., Pugliese, G., Corigliano, G., Balducci, S. (2010). Exercise for the management of type 2 diabetes: a review of the evidence. *Acta Diabetol*, 47, 15-22.

Zeng, H., Irwin, M.L., Lu, L., Risch, H., Mayne, S., Mu, L., Deng, Q., Scarampi, L., Mitidieri, M., Katsaros, D., Yu, H. (2012). Physical activity and breast cancer survival: an epigenetic link through reduced methylation of a tumor suppressor gene L3MBTL1. *Breast Cancer Res Treat*, 133, 127-135.

Zentner, G.E., Henikoff, S. (2013). Regulation of nucleosome dynamics by histone modifications. *Nat Struct Mol Biol*, 20, 259-266.

Zhang, F.F., Cardarelli, R., Carroll, J., Zhang, S., Fulda, K.G., Gonzalez, K., Vishwanatha, J.K., Morabia, A., Santella, R.M. (2011). Physical activity and global genomic DNA methylation in a cancer-free population. *Epigenetics*, 6, 293-299.

Zhang, L., Zhang, J., Jiang, Q., Zhang, L., Song, W. (2018). Zinc binding groups for histone deacetylase inhibitors. *J Enzyme Inhib Med Chem*, 33, 714-721.

Zheng, J., Liu, L., Wang, S., Huang, X. (2015). SUMO-1 Promotes Ishikawa Cell Proliferation and Apoptosis in Endometrial Cancer by Increasing Sumoylation of Histone H4. *Int J Gynecol Cancer*, 25, 1364-1368.

977 Zimmer, P., Baumann, F.T., Bloch, W., Schenk, A., Koliamitra, C., Jensen, P., Mierau, A.,
978 Hulsdunker, T., Reinart, N., Hallek, M., Elter, T. (2014). Impact of exercise on pro
979 inflammatory cytokine levels and epigenetic modulations of tumor-competitive lymphocytes
980 in Non-Hodgkin-Lymphoma patients-randomized controlled trial. *Eur J Haematol* , 93, 527-
981 532.
982 Zimmer, P., Bloch, W., Schenk, A., Zopf, E.M., Hildebrandt, U., Streckmann, F., Beulertz, J.,
983 Koliamitra, C., Schollmayer, F., Baumann, F. (2015). Exercise-induced Natural Killer Cell
984 Activation is Driven by Epigenetic Modifications. *Int J Sports Med*, 36, 510-515.