International Journal of Molecular Medicine & Advance Sciences
MEDWELL PUBLICATIONS

A Review on RNA-Interference, Non-Coding-RNA, Micro-RNA, Gene Regulation and Gene Doping

Alemayehu Choramo
College of Agriculture and Natural Resource, Bonga University, Bonga, Ethiopia

Key words: Doping, genes, interference, MicroRNA, non-coding RN

Corresponding Author:
Alemayehu Choramo
College of Agriculture and Natural Resource, Bonga University, Bonga, Ethiopia

Page No.: 5-13
Volume: 15, Issue 2, 2019
ISSN: 1813-176x
International Journal of Molecular Medicine and Advance Sciences

Abstract: Many physiological activities take place in the cells of living organisms. These cellular activities are controlled by the genes of the cell. The activities of DNA, RNA and proteins are indispensable for living things. However, the importance of RNA has long been in the shadow of other cellular components, DNA and proteins. Later as the time progress on, this was experimentally demonstrated revealing that during gene expression, DNA is copied in a molecule of messenger RNA (mRNA) that is then translated into proteins molecules. Ribonucleic acid (RNA) is classified in to different categories based on their functions and sizes. Therefore, objectives this review is: to understand the mechanism how RNA interferes, how non-coding RNA regulates gene expression, to understand concept of gene doping and related effects and to understand MicroRNAs and their role for viral diagnosis. The term non-coding RNA (ncRNA) is commonly employed for RNA that does not encode a protein. Cells of living things are self-regulated by the means of interactions between different molecules. RNAs with regulatory function in most cases by base pairing with complementary sequences in other RNAs or DNA, forming RNA: RNA and probably RNA: DNA complexes that are recognized and acted upon by a relatively generic infrastructure (such as RNA-induced silencing complex (RISC) complexes or RNA editing enzymes). On the other hand, RNA interference (RNAi) is a form of Post-Transcriptional Gene Silencing (PTGS) which is regarded as a mechanistic variant of RNAi. MicroRNAs are a group of small, non-coding RNAs which regulate the process of gene expression in a sequence-specific manner. MicroRNAs plays a role in disease diagnosis due to their effort in apoptosis, proliferation of cell, differentiation and development. In generally sense, gene doping is could be defined as ‘‘the use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance for non-therapeutic purposes.’’

INTRODUCTION

Many different biological activities take place in the cells of living organisms. These cellular activities are controlled by the genes of the cell. However, the role of RNA has long been hidden in the shadow of DNA and proteins molecules. But later, in the late 1800's the importance and function RNA has been understood. In the 1950s, when the molecular structure of DNA established, it was proposed that RNA molecules would be an intermediate molecule in the information flux between DNA and proteins. Later on, this was experimentally demonstrated revealing that during gene expression, DNA is copied in a molecule of messenger RNA (mRNA) which is then translated into proteins with the help of other RNA molecules such as transfer RNA (tRNA) and ribosomal RNAs (rRNAs) (Gomes *et al*., 2013).

Ribonucleic acid molecule, got its name from the sugar group in the molecule's backbone-ribose. The primary structure of both RNA and DNA is similar. However, several important some differences exist between RNA and DNA. Like DNA, RNA has a sugar-phosphate backbone with nucleotide bases attached to it. Like DNA, RNA contains the bases adenine (A), cytosine (C) and guanine (G) but, RNA does not have thymine, instead, contain uracil (U) base. Unlike DNA molecule, RNA is a single-stranded molecule its base composition does not follow Chargaff's rule. Nevertheless, there is some degree of secondary structure in the different RNA types because the molecule can form hairpin loops of hydrogen bonded A-U or G-C pairs. The real sequence of ribo-nucleotides in RNA is commonly called its primary structure. When the loops are included it is said to have a secondary structure. It can also fold into a three dimensional shape termed as its tertiary structure (Gomes *et al*., 2013).

Ribonucleic acid (RNA) is classified in to different categories based on their functions and sizes. For example, ribosomal RNA (rRNA) is structural and functional components of ribosomes, messenger RNA (mRNA) encode proteins, transfer RNA (tRNA) plays major role in the process of translation. Non-coding RNA (ncRNA) is mostly employed for RNA that does not encode a protein and a number of well-characterized antisense transcripts that appear to play regulatory functions in relation to their sense gene, small RNA (sRNA) molecules that base pair with the mRNA and regulates gene expression post-transcriptionally (Storz *et al*., 2005), dsRNA induces degradation of the homologous mRNA, mimicking the effect of the reduction, or loss, of gene activity which result gene regulation (Pasquinelli and Ruvkun,2002), rRNAs and tRNAs involved in mRNA translation, small nuclear RNAs (snRNAs) involved in splicing and in the modification of rRNAs (Mattick and Makunin, 2006). Furthermore, their activities in various aspects like interference, disease diagnosis, gene regulation, doping effects and non-coding activities are discussed widely in this review notes.

Objectives: To understand the mechanism how RNA interfere, how non-coding RNA regulate gene expression. To understand concept of gene doping and related effects. To understand microRNAs and their role for viral diagnosis.

NON-CODING RNA

Generally the term non-coding RNA (ncRNA) is mostly employed for RNA that does not encode a protein, however this does not mean that such RNAs do not contain information or nor have function. Most of the ncRNAs identified in genomic transcriptome studies have not been studied and have yet to be attributed any role. However, there are many lines of evidence of studies that suggest that these RNAs are biologically meaningful (Mattick and Makunin, 2006).

First, most intensively studied gene loci, including both those that are imprinted and classical loci such as beta-globin have been indicated to express non-coding transcripts. This includes some enhancers and conserved region of intergenic sequences (Ashe *et al* , 1997). Second, it is obvious thatmost of these transcripts are cell type specific with specific subcellular regions and are developmentally regulated (Blackshaw *et al*., 2004). A huge number of ncRNAs are specifically expressed from either the paternal or the maternal allele at imprinted loci and some are related with human diseases such as the Prader-Willi and Angel man syndromes (Morison *et al*., 2005). Hence, the genetic cause for some and perhaps many, diseases may be associated with mutations within ncRNAs (Le Meur *et al*., 2005). Third, the upstream regions of ncRNA transcripts show most of the features normally related with promoters (Cawley *et al*., 2004) and, may be more highly conserved than the promoters of protein-coding genes (Carninci *et al*., 2005). The human genome has comparable numbers of protein-coding and non-coding genes that are bound by classical transcription factors and regulated by common environmental signals (Cawley *et al*., 2004). Finally, a huge number of ncRNAs have been shown to be functional including the well-characterized ncRNAsXist and Tsix that control X-chromosome inactivation in mammals (Chureau *et al*., 2002). They also include a number of well-characterized antisense transcripts that appear to play regulatory function in relation to their sense gene including those opposite FGF-2 (fibroblast growth factor-2), HIF-1 (hypoxia inducible factor-1) and myosin heavy chain (Werner, 2005).

Although, it has been generally considered that most genetic information is transacted by proteins, recent study evidence suggests that the most of the genomes of mammals and other complex living things is indeed transcribed into ncRNAs, most of which are alternatively spliced and/or processed into smaller products. These ncRNAs include microRNAs and small nuclear RNAs (snoRNAs), many of which remain to be identified as well as likely other classes of yet to be revealed small regulatory RNAs and tens of thousands of longer transcripts (including complex patterns of interlacing and overlapping sense and antisense transcripts), most of whose role are unknown. These RNAs (including those derived from introns) appear to encompass a hidden layer of internal signals that regulate various levels of gene expression in physiology and development including chromatin architecture/epigenetic memory, transcription, RNA splicing, editing, translation and return. The RNA regulatory networks may determine most of our complex characteristics play a significant role in disease and constitute an unexplored world of genetic variation both within and between species (Mattick and Makunin, 2006).

Non-coding RNAs play a part in a surprisingly different collection of regulatory events, ranging from copy number regulates in bacteria to X-chromosome inactivation in mammals. MicroRNAs (miRNAs) are a member of 21-25-nucleotide small RNAs which at least for those few which have characterized targets, negatively regulate gene expression at the post-transcriptional level 3-5. Members of the miRNA folks were initially discovered as small temporal RNAs (stRNAs) which control developmental transitions in Caenorhabditis elegans (Pasquinelli and Ruvkun, 2002). Until in recent times most of the known non-coding RNAs (ncRNAs) fulfilled relatively generic role in cells such as the rRNAs and tRNAs involved in mRNA translation, small nuclear RNAs (snRNAs) are involved in splicing and small nucleolar RNAs (snoRNAs) involved in the modification of rRNAs (Mattick and Makunin, 2006).

Gene regulation: All living organisms are self-regulated by interactions between diverse molecules. Until very recently; many research has focused on transcription regulation interactions and on protein-protein interactions, that in many cases are involved in post-translational regulation. During the last years it has become obvious that another type of interaction plays a important role in the regulation of cellular processes, manifested by small RNA (sRNA) molecules that base pair with the mRNA and regulates gene expression post-transcriptionally. This mode of regulation was originated in both pro-and eukaryotes (Storz *et al*., 2005).

Although, there are differences in the character of the eukaryotic and prokaryotic regulatory RNAs and in the fine-details of their means of action, both exert their regulatory function mostly by base pairing with the mRNA and influencing translation or mRNA solidity (Shimoni *et al*., 2007).

Regulatory RNAs role in most cases by base pairing with complementary sequences in other RNAs and DNA, to form RNA: RNA (and probably RNA: DNA) complexes that are known and acted upon, by a relatively generic infrastructure (such as RNA-induced silencing complex (RISC) complexes or RNA editing enzymes). There are a lot of well characterized examples of regulatory RNA sequences in the untranslated regions (UTRs) of mRNAs that act in cis as receivers of other trans-acting signals, by forming secondary structures which combine regulatory proteins or small molecular weight ligands. Examples of the former include sequences in UTRs that can bind regulatory proteins or be the targets ofRNA editing to control the stability, translatability or localization of mRNAs (Gebauer and Hentze, 2004). The base pairing blocks the binding of the ribosome to the mRNA, thus, negatively regulating translation (Levine *et al*., 2007).

RNA INTERFERENCE

RNA interference (RNAi) is a means of post-transcriptional gene silencing, in which dsRNA provokes dilapidation of the homologous mRNA, mimicking the cause of the reduction or loss, of gene action (Pasquinelli and Ruvkun, 2002). The introduction into the cell of dsRNA (double-stranded RNA) containing nucleotide sequence complementary to an mRNA sequence causes discerning degradation of the latter and thus silencing of a specific gene. This incident, known as RNA interference (RNAi) which is supposed to be present in nearly all eukaryotic organisms. The RNAi is also ableto silencing of transposons in germ line cells and struggle RNA virus infection. Some enzymes concerned in this practice show high homology across species. Some of these enzymes are involved in other cellular processes, for instance developmental timing, suggesting strong interconnections between RNAi and other metabolic pathways. The RNAi is most likely an ancient mechanism that evolved to guard eukaryotic cells against invasive forms of nucleic acids (Szweykowska-Kulinska *et al*., 2003). RNAi is a method, similar to Post-Transcriptional Gene Silencing (PTGS) in plants and quelling in Neurospora, for decreasing the expression of a target gene by the processing of double-stranded RNA precursors to 22nt guide RNAs, called small interfering RNAs (siRNAs) (Bernstein *et al*., 2001).

RNAi can be classified into four different stages. Those four stages are: double-stranded RNA cleavage, silencing complex formation, silencing complex activation and mRNA degradation (Martinez *et al*., 2002).

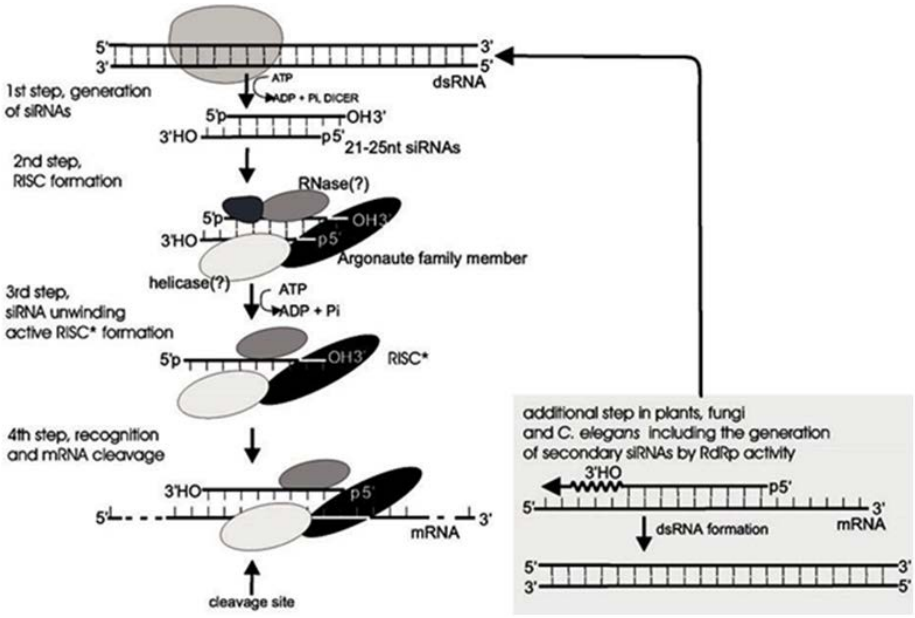

Fig. 1: Schematic representation of four-step gene silencing pathway; (Szweykowska-Kulinska *et al*., 2003)

The first one includes ATP-dependent, processive dsRNA cleavage into double-stranded fragments 21-25 nucleotides elongated. They contain 5-phosphate and 3-hydroxyl terminus and two additional overhanging nucleotides on their 3 ends (El-Bashir *et al*., 2001). The generated fragments were called small interfering RNAs (siRNAs). It was revealed that the already mentioned structural features of siRNAs are vital for the next stages of the RNAi pathway, e.g., modification of the 5 end of the antisense strand inhibits siRNA activity, whereas blunt-ended siRNAs are very inefficient intermediates in further steps (Martinez *et al*., 2002) (Fig. 1).

Quelling: Much before than RNAi discovery, trans-gene-induced gene silencing was observed in the filamentous fungus *N. crassa*. The occurrence is known quelling, it affected both transgenes and endogenous genes. The filamentous fungus *Neurospora crassa* is a model organism for the study of gene silencing. The nearly all characterized gene silencing mechanism in this ascomycete is quelling which occurs at the post-transcriptional level. Quelling is triggered by the introduction of transgenes and results in silencing of both transgenes and similar endogenous mRNAs. Quelling is associated to co-suppression, observed in plants and RNA interference in animals; it requires an Argonaute protein and acts by generating small RNA molecules (about 25 nt long) which in turn target mRNAs to be silenced. It has been recently revealed that quelling is needed for the taming of transposons but unlike other model organisms, does not look like to play any role in heterochromatin assembly and maintenance (Fulci and Macino., 2007).

Post Transcriptional Gene Silencing (PTGS): Post Transcriptional Gene Silencing (PTGS) is regarded as a mechanistic variation of RNAi. In broad sense, this term defines both the co-suppression of an endogenous gene by the introduction of a transgene into the nuclear plant genome and the cellular defense mechanism induced by RNA virus infection (Szweykowska-Kulinska *et al*., 2003). As we discussed above, in the case of quelling in fungi and RNAi in animals, PTGS operates via. siRNAs and their buildup requires either transgene sense or antisense transcription or RNA virus replication (Hamilton and Baulcombe, 1999). Post Transcriptional Gene Silencing (PTGS) induced locally spreads systemically to other tissues of the plant. Such a process requires the presence of a signal molecule transporting information about PTGS triggering. SiRNAs were originating in both the site of silencing initiation and in distal tissues. Although, the nature of the signaling molecule leftovers unidentified, siRNAs are small sufficient to move through plasmodesmata and/or via. the vascular system. As a result, at present siRNAs are the finest candidates for signal molecules ensuring total spreading and specificity of PTGS. Total gene silencing in plants was investigated using grafting process. It was revealed that silencing was transmitted from silenced stocks to non-silenced scions. Spread was unidirectional, always from stock to scion, transgene specific and locus independent and required a transcriptionally active transgene in the target scion (Palauqui *et al*., 1997).

Enzymes involved in RNAi: Enzymes concerned in this course show high homology across species. A number of

these enzymes are involved in other cellular processes, for instance developmental timing, signifying strong interconnections between RNAi and other metabolic pathways (Szweykowska-Kulinska *et al*., 2003). A huge number of genes whose products are some way concerned in RNA silencing have been recognized in *C. elegans, D. melanogaster*, *Homo sapiens*, *Dictyostelium discoideum*, *N. crassa*, *Chlamydomonas reinhardtii* and *A. thaliana*. The identified genes encode dsRNases, RNA-dependent RNA polymerases, RNA-dependent helicases and proteins of unidentified function. The enzyme first discovered in Drosophila and called Dicer has dsRNase activity and is involved in the first step of RNA silencing the production of siRNAs. It belongs to the RNase III-family. Dicer is ATP-dependent and contains several characteristic domains: an N-terminal helicase domain, a PAZ domain (a domain conventional all over evolution found in Piwi/Argonaute/Zwille proteins in Drosophila and Arabidopsis and involved in developmental control), dual RNase III do-mains and a double stranded RNA-binding domain (Bernstein *et al*., 2001). A homologue of this gene was found and characterized in C. elegans-dcr-1 (Ketting *et al*., 2001).

MICRO-RNA

MicroRNAs are a family of small, non-coding RNAs that control gene expression in a sequence-specific mode. The two founding members of the microRNA family were originally recognized in *Caenorhabditis elegans* as genes that were required for the timed regulation of developmental activities. Since then, hundreds of MicroRNAs have been identified in approximately all metazoan genomes, together with worms, flies, plants and mammals. MicroRNAs have varied expression mode and might regulate various developmental and physiological activities (Mukhadi *et al*., 2015). Their detection adds a new aspect to understanding of complex gene regulatory networks. MicroRNA (miRNA or miRs) are tiny regulatory RNAs of approximately 19-25 nucleotides piece and are involved in post-transcriptional gene silencing in the entire eukaryotes. They were revealed in 1993 in the nematode *Caenorhabditis elegans* where they were shown to play crucial roles in gene regulatory networks. Their importance remained undervalued due to their extraordinary characteristics and unidentified function at that time. After the discovery of the first miRNA, lin-4, in the nematode *C. elegans*, a second important miRNA, let-7 was as well identified in the same being in 2000 (Reinhart *et al*., 2000). MicroRNAs (miRNAs) regulate pathophysiological events that repress gene expression by binding to messenger RNAs. These biological molecules could be used to study gene regulation and protein expression that will allow better understanding of many cellular activities such as cell cycle progression and apoptosis that control the outcome of cells (Mukhadi *et al*., 2015).

Micro-RNA biogenesis: Matured miRNAs are evolutionarily preserved single-stranded RNAs. Several miRNAs are encoded by genomic regions which are situated within introns and intergenic regions of non-coding RNAs (Reinhart *et al*., 2000). The biogenesis of miRNAs starts in the nucleus, where the miRNA gene is transcribed by RNA polymerase II or III, to make a long primary miRNA (Fig. 2). The MiRNA biogenesis path requires two RNase III enzymes, Drosha and Dicer. Drosha processes the principal miRNA transcript

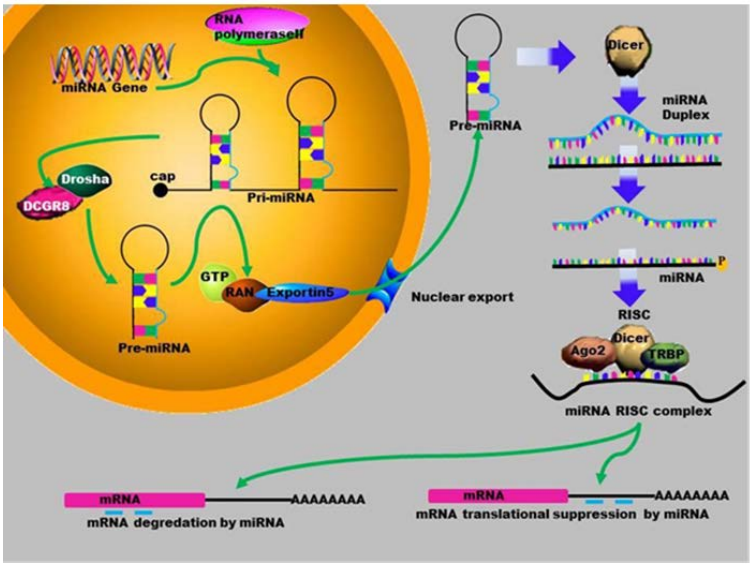

Fig. 2: Schematic outline of the biogenesis of miRNA; (Mukhadi *et al*., 2015)

(pri-miRNA) into a ~60-100 nucleotides hairpin structure known as the precursor-miRNA (pre-miRNA). MiRNAs are elated out of the nucleus by Exportin-5 to the cytoplasm where another RNase III, Dicer, further processes them, into mature, 22 base pair nucleotides. One strand is degraded while the other one binds to the 3'-untranslated region of the target messenger RNA (mRNA). This complex is composed of several proteins that include the Argonaute proteins which allow a stable conservation of the miRNA. The interaction between the miRISC complex and the mRNA could also have a direct consequence on protein translation. Nowdays, several studies have revealed that miRNAs also play an important role in gene activation (Mukhadi *et al*., 2015).

Mi-RNA is transcribed by RNA polymerase II to form a primary MicroRNA molecule. These Pri-mRNA molecules are big RNA precursors and are comprised of a 5'-cap and poly-A tail. This is then cleaved by the Drosha complex to generate pre-miRNA that is exported to the cytoplasm by Exportin-5 where it is processed by Dicer into a miRNA duplex. The guide strand (mature miRNA) is then integrated into the miRISC where gene silencing could be accomplished through mRNA target cleavage (degradation) or through the avoidance of translation.

Important MicroRNAs: A number of of vital MicroRNAs based on diverse biological activities they involved are MiR-21, MiR-29 and miR-200c. MiR-21 is not specific to a certain diseases and is believed to play an important role in the development of numerous malignancies such as cancer and kidney diseases. As a result, it is not surprising that miR-21 is involved in different biological activities including cell differentiation, proliferation and apoptosis. MiR-21 has been reported to be more expressed in kidney diseases (Mukhadi *et al*., 2015). MiR-29: Groups of the miRNA 29 family are commonly-down regulated in the majority types of cancers, although, there are exceptions. Commonly, they regulate the expression of proteins such as collagens, transcription factors and methyltransferases. A current study verified that increasing miR-29a levels protects cells against diabetic podo-cytopathy by suppressing HDAC4 signaling, nephron ubiquitination and urinary nephrin excretion related with diabetes, as well as restoring nephron acetylation (Schmitt *et al*., 2013). MiR-200c has been reported that miR-200c suppresses ZEB1 and ZEB2 and also regulates induction of apoptosis through the death receptor CD95. This allows members of the miR-200 family to avoid TGF-ß-mediated epithelial-mesenchymal transition (EMT) (Mukhadi *et al*., 2015).

MiRNA-viral disease diagnosis: MiRNAs are implicated as playing a role in diseases diagnosis due to their significance in apoptosis, cell proliferation, differentiation and development. As miRNAs have been detected in a stable condition in diverse biological fluids, they have the capacity to be tools to study the pathogenesis of human diseases with a large potential to be used in disease prognosis and diagnosis. Renal diseases have been more and moredetected as the most important common complication of Human Immunodeficiency Virus (HIV) infection worldwide (Mukhadi *et al*., 2015).

One of the most common kidney diseases in patients with HIV/AIDS is Human Immunodeficiency Virus-Associated Nephropathy (HIVAN) which is an End-Stage Renal Disease (ESRD). HIVAN was initially detected in patients with Acquired Immunodeficiency Syndrome (AIDS) and was formerly known as AIDS-associated nephropathy. However, the name was altered to HIVAN when renal histological features alike to those observed in patients with full-blown AIDS were observed in asymptomatic persons. HIVAN could be defined as an destructive form of focal segmental glomerulosclerosis (FSGS) characterized by fail of the glomerular tuft and related tubule interstitial lesions and develops late in the course of HIV-1 infection following the development of AIDS (Lucas *et al*., 2008).

The amounts of certain miRNAs have been observed to increase or decrease during HIV infection indicating that miRNAs play a great role in HIV related diseases. Moreover, the deregulation of miRNAs during HIV infection has been observed to play a vital role in disease occurrence and progression. Different studies have shown that deregulation of miRNAs due to HIV-1 infection plays a crucial role in disease development and revealed that miRNAs may be early signs of host cellular dysfunction induced by HIV-1. In one such pathology, HIV encephalitis (HIVE), changes in miRNA regulation led to an increase in the levels of apoptosis contributing to the disease pathology (Mukhadi *et al*., 2015).

GENE DOPING (PAST, PRESENT AND FUTURE)

In general term gene doping is defined as ''the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance.'' The International Olympic Committee has integrated the technique of gene doping in their list of banned classes of substances and prohibited methods. The prohibited catalog of as available by the World Anti-Doping Agency (WADA, 2013), yet includes the method of gene doping. Gene doping has a lot of aspects. It is, for example, imaginable that once genetic therapies have become routine in regular medicine, they may be very valuable and tolerable for the athletic world as well (e.g., to treat injuries). On the other hand, the probable to abuse such therapies to improve athletic performance in otherwise perfectly healthy individuals can be considered a threat (Lemkine and Demeneix, 2001).

Gene delivery or introducing: Genetic matter can be inserted into a cell in either *vivo* or *ex-vivo*. The former (*in vivo*) strategy is direct gene insertion into the human body, i.e., into main blood vessels or the target tissue/organ. In the latter (*ex-vivo*) or indirect DNA transfer strategy, i.e., *ex vivo* gene delivery, cells are collected from the body of the patient and then, after genetic modification, breeding and selection, are reintroduced into the patient's body (Brzezianska *et al*., 2014).

In gene therapy or in gene doping the genetic material is delivered into cells and tissues using a variety of carriers that can be viral or non viral. Using viral vectors (attenuated retroviruses, adenoviruses or lent viruses) a transgene is released in target cells and is expressed using cell replication machinery. Some of these viruses, such as retroviruses, integrate their genetic material with chromosomes of a human cell. Other viruses, such as adeno-viruses, introduce the transgene into the cell nucleus without chro-mosomal integration viral vectors are efficient gene delivery carriers and they offer several advantages: large packaging capacity, cell-specific tropism and/or long-term expression (Lundstrom and Boulikas, 2003).

Non viral gene delivery methods are less effective methods of introducing genetic material into human cells, though characterized by low cytotoxicity. These include physical methods such as elec-troporation, "gene gun" and chemical carriers using cationic liposomes or biodegradable polymers (polyethylenimines; PEIs). Non-viral gene delivery systems may cause an increased immune response (Lemkine and Demeneix, 2001).

Gene doping in the past: In the past few years, significant progress concerning the knowledge of the human genome map has been achieved. Consequently, attempts to use gene therapy in patients' management are more and more often undertaken. The major aim of gene therapy was to substitute malfunctioning genes *in vivo* and/or to promote the long-term endogenous synthesis of scarce protein. *In vitro* studies enhanced the production of human recombinant proteins such as insulin (INS), Growth Hormone (GH), insulin-like growth factor-1 (IGF-1) and erythropoietin (EPO) which could have therapeutic application (WADA, 2013). Doping, although, prohibited by the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA), has been used since the early on 1920s, in the form of, among others, anabolic steroids, erythropoietin, amphetamine and modafinil (Brzezianska *et al*., 2014).

Gene doping in the present: Unluckily, genetic techniques developed for therapeutic purposes are increasingly being used in competitive sports. A few new substances (e.g., antibodies against myostatin or myostatin blockers) may be used in gene doping in athletes. The use of these substances may cause araise of body weight and muscle mass and a significant improvement of muscle strength. Although, it is confirmed that unregulated manipulation of genetic material and/or the insertion of recombinant proteins may be related to health risks, athletes are increasingly turning to prohibited gene doping (Brzezianska *et al*., 2014). Now, with the completion of the Human Genome Project (HUGO Project) and the development of gene therapy in medicine, there has been energetic advancement of research on gene doping and gene delivery technologies to improve athletic performance in a variety of sports (WADA, 2013) .

Gene doping in the future: At the same time, anti-doping research is undergone in various laboratories around the world to try to develop and filter ever newer techniques for gene doping revealing in sport. Thanks to the World Anti-Doping Agency (WADA) and some other sports organizations there is a hope for actual guard of athletes from adverse health effects of gene doping which at the same time gives a opportunity to sustain the idea of fair play in sport (Lemkine and Demeneix, 2001). Another problem is still not accomplished work on the standardization of consistent tests to detect gene doping. Therefore, the scientific and medical communities should support the activities of the World Anti-Doping Agency (WADA) in developing new methods of gene doping detection and updating the lists of prohibited agents. In addition to educational programs for athletes and development of tests for gene doping detection, an individual means of gene doping control should be introduced; in which each athlete would be the self-reference baseline (WADA, 2013).

Advantage of gene doping: The reason of gene doping is indeed to specifically modify endogenous gene activity, whether this is in the form of the activation, strengthening, weakening or blocking of so-called gene expression. The underlying biochemical and physiological activities are highly complex, both at the cellular level and at the level of on the whole regulation in the body (and thus, will only be outlined in the report). The network of response controls of attributes relevant to physiological performance results in a multitude of targets for pharmacological and molecular biological modulation for new therapeutic treatment AIMES but also in turn for doping purposes. The possible consequences of such interventions are very hard to predict. This can still be, seen when used in medical therapy to try to treat patients (in the form of adverse outcomes or a shortage of efficacy of the agents) (Gerlinger *et al*., 2008).

Side effects of gene doping: The risks involved in gene doping are numerous and are associated with the vector

used (chemical and viral) and related to the encoded transgene. Yet, gene therapy has been comparatively safe; thousands of patients have been treated in well-controlled clinical gene therapy trials with pharmaceutical grade gene therapy vectors and have revealed few side effects. The therapy is limited to the patient with no transmission to offspring or next of kin. With gene doping, gene transfer vectors may be produced in non-controlled laboratories. These preparations might be contaminated with chemicals and other impurities from the production and refining process including pyrogens. Virulent viral gene therapy vectors may be formed which poses a major safety concern. In the case of virulent viruses, these are not only risky to the athlete but also pose a health risk for the general population who might get infected. Health risks consequential from expressed genes are similar to those of other doping forms. However, the level and duration of protein production is less controllable when compared to predictable protein administration. For example, EPO delivered by gene therapy could result in sustained high EPO levels which would increase the chances of stroke and heart attack (Haisma *et al*., 2004).

CONCLUSION

Generally, the term non-coding RNA (ncRNA) is normally employed for RNA that does not encode a protein. Regulatory RNAs function in nearly all cases by base pairing with complementary sequences in other RNAs and DNA, to form RNA: RNA and probably RNA: DNA complexes. RNA interference (RNAi) is a form of post-transcriptional gene silencing, in which dsRNA causes breakdown of the homologous mRNA. MicroRNAs are a family of small, non-coding RNAs that control gene expression. The levels of certain miRNAs have been observed to flactuate during HIV infection indicating that miRNAs play animportant role in viral diagnosing. Gene doping is the non-therapeutic use of genes, genetic elements and/or cells by athletes. Gene doping change (modify) endogenous gene activity. The risk concerned in gene doping is numerous and are related to the vector used (chemical and viral) and associated with the encoded transgene. Based on the above conclusions the following recommendations are forwarded:

- Further researches should be done to investigate alternative vectors as chemical and viral vectors risk full in gene introducing
- World Anti-Doping Agency (WADA) should work strong to avoid gene doping in athletics and other sports

REFERENCES

Ashe, H.L., J. Monks, M. Wijgerde, P. Fraser and N.J. Proudfoot, 1997. Intergenic transcription and transinduction of the human ß-globin locus. Genes Dev., 11: 2494-2509.

Bernstein, E., A.M. Denli and G.J. Hannon, 2001. The rest is silence. RNA, 7: 1509-1521.

Blackshaw, S., S. Harpavat, J. Trimarchi, L. Cai and H. Huang *et al*., 2004. Genomic analysis of mouse retinal development. PLoS Biol., 2: E247-E247.

Brzezianska, E., D. Domanska and A. Jegier, 2014. Gene doping in sport-perspectives and risks. Biol. Sport, 31: 251-259.

Carninci, P., T. Kasukawa, S. Katayama, J. Gough and M.C. Frith *et al*., 2005. The transcriptional landscape of the mammalian genome. Science, 309: 1559-1563.

Catalanotto, C., G. Azzalin, G. Macino and C. Cogoni, 2002. Involvement of small RNAs and role of the qde genes in the gene silencing pathway in Neurospora. Genes Dev., 16: 790-795.

Cawley, S., S. Bekiranov, H.H. Ng, P. Kapranov and E.A. Sekinger *et al*., 2004. Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. Cell, 116: 499-509.

Chureau, C., M. Prissette, A. Bourdet, V. Barbe and L. Cattolico *et al*., 2002. Comparative sequence analysis of the X-inactivation center region in mouse, human and bovine. Genome Res., 12: 894-908.

El-Bashir, S.M., W. Lendeckel and T. Tuschl, 2001. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev., 15: 188-200.

Fulci, V. and G. Macino, 2007. Quelling: Post-transcriptional gene silencing guided by small RNAs in *Neurospora crassa.* Curr. Opin. Microbiol., 10: 199-203.

Gebauer, F. and M.W. Hentze, 2004. Molecular mechanisms of translational control. Nat. Rev. Mol. Cell Biol., 5: 827-835.

Gerlinger, K., T. Petermann and A. Sauter, 2008. Gene doping. Working Report No. 124, The Office of Technology Assessment at the German Bundestag is an Independent Scientific Institution, German.

Gomes, A.Q., S. Nolasco and H. Soares, 2013. Non-coding RNAs: Multi-tasking molecules in the cell. Int. J. Mol. Sci., 14: 16010-16039.

Haisma, H.J., O. De Hon, P. Sollie and J. Vorsten, 2004. Gene doping. Netherlands Centre for Doping Affairs (NeCeDo), Netherlands.

Hamilton, A. and D.C. Baulcombe, 1999. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science, 286: 950-952.

Ketting, R.F., S.E. Fischer, E. Bernstein, T. Sijen, G.J. Hannon and R.H. Plasterk, 2001. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. Elegans. Genes Dev., 15: 2654-2659.

Le Meur, E., F. Watrin, M. Landers, R. Sturny, M. Lalande and F. Muscatelli, 2005. Dynamic developmental regulation of the large non-coding RNA associated with the mouse 7C imprinted chromosomal region. Dev. Biol., 286: 587-600.

Lemkine, G.F. and B.A. Demeneix, 2001. Polyethylenimines for *in vivo* gene delivery. Curr. Opin. Mol. Ther., 3: 178-182.

Levine, E., Z. Zhang, T. Kuhlman and T. Hwa, 2007. Quantitative characteristics of gene regulation by small RNA. PLoS Biol., Vol. 5, No. 9. 10.1371/journal.pbio.0050229

Lucas, G.M., B. Lau, M.G. Atta, D.M. Fine, J. Keruly and R.D. Moore, 2008. Chronic kidney disease incidence and progression to end-stage renal disease, in HIV infected individuals: A tale of two races. J. Infect. Dis., 197: 1548-1557.

Lundstrom, K. and T. Boulikas, 2003. Viral and non-viral vectors in gene therapy: Technology development and clinical trials. Technol. Can. Res. Treat., 2: 471-485.

Martinez, J., A. Patkaniowska, H. Urlaub, R. Luhrmann and T. Tuschl, 2002. Single stranded antisense siRNA guide target RNA cleavage in RNAi. Cell, 110: 563-574.

Mattick, J.S. and I.V. Makunin, 2006. Non-coding RNA. Hum. Mol. Genet., 15: R17-R29.

Morison, I.M., J.P. Ramsay and H.G. Spencer, 2005. A census of mammalian imprinting. Trends Genet., 21: 457-465.

Mukhadi, S., R. Hull, Z. Mbita and Z. Dlamini, 2015. The role of microRNAs in kidney disease. Non-Coding RNA, 1: 192-221.

Palauqui, J.C., T. Elmayan, J.M. Pollien and H. Vaucheret, 1997. Systemic acquired silencing: Transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J., 16: 4738-4745.

Pasquinelli, A.E. and G. Ruvkun, 2002. Control of developmental timing by microRNAs and their targets. Annu. Rev. Cell Dev. Biol., 18: 495-513.

Reinhart, B.J., F.J. Slack, M. Basson, A.E. Pasquinelli and G. Ruvkun *et al*., 2000. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature, 403: 901-916.

Schmitt, M.J., C. Margue, I. Behrmann and S. Kreis, 2013. MiRNA-29: A microRNA family with tumor-suppressing and immune-modulating properties. Curr. Mol. Med., 13: 572-585.

Shimoni, Y., G. Friedlander, G. Hetzroni, G. Niv, S. Altuvia, O. Biham and H. Margalit, 2007. Regulation of gene expression by small non-coding RNAs: A quantitative view. Mol. Syst. Biol., Vol. 3, No. 1.

Storz, G., S. Altuvia and K.M. Wassarman, 2005. An abundance of RNA regulators. Annu. Rev. Biochem., 74: 199-217.

Szweykowska-Kulinska, Z., A. Jarmolowski and M. Figlerowicz, 2003. RNA interference and its role in the regulation of eucaryotic gene expression. Acta Biochim. Pol., 50: 217-229.

WADA., 2013. The world anti-doping code: The 2013 prohibited list international standard. World Anti-Doping Agency, Montreal, Canada.

Werner, A., 2005. Natural antisense transcripts. RNA Biol., 2: 53-62.