

**Ribonucleic acid interference,
the cure for canine
obesity?**

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Abstract

In this paper, I am going to outline a possible method of reducing the amount of canine obesity in the UK, using a method called RNA interference, which basically stops the gene from being expressed in the cells. I will also discuss the ethical implications surrounding the concept of RNA interference.

Introduction

Proteins are the key structural building blocks in the body, they are used for structural purposes like collagen, or for metabolic functions, carried out by globular proteins, such as enzymes and haemoglobin. Proteins are coded for by genes, located on chromosomes in DNA, some genes are desirable, others aren't. RNA interference is a way of silencing the undesirable genes without affecting the other genes on the chromosome.

Since being discovered in 1998, RNA interference is thought to be one of the greatest discoveries in the past couple of decades. It is thought that grasping the concept of RNA interference could be the answer to many of the medical problems facing the world today, for example genetic disorders which cause conditions like cardiomyopathy, or cancer. Current in vitro studies have shown that RNAi is efficient in viral diseases, such as HIV, influenza and the human papillomavirus infection. It has also been proven efficient in some neurodegenerative diseases, such as parkinsons and Alzheimers, and in cancer.

RNA interference works in vertebrates by triggering an interference response, but not an inflammatory response, the inflammatory response is undesirable as it stops the production of all proteins, which would cause considerable damage to the organism.

The process of RNA interference is very much like the natural occurring process in which T lymphocytes in the body remember and destroy certain pathogens, but RNA interference can go one step further and stop something undesirable happening in the body, before it happens. It was popularised by work in worms by Richard Fire and Craig Mello in 1998, when long double stranded RNAs were injected into a worm's gonad. Injection into the gland is a standard way of introducing trans-genes into worms. Trans-genes blocked the expression of endogenous genes in a way which was sequence specific. In Eukaryotic organisms most protein coding genes are transcribed by RNA polymerase 2, which creates pre-mRNAs which are processed to form mature mRNAs. These mRNAs are then transported from the nucleus across the cytoplasm, where they are translated in the ribosomes.

RNAi can regulate gene expression and can be activated by short regulatory RNAs known as microRNAs. In worms and flies, RNAi can be activated by endogenous genetic mutation. In plants and cultured insect cells RNAi plays a role in antiviral defence, in which viral double stranded RNAs are targeted for destruction by RNAi.

When long double stranded RNAs enter a cell they are recognised and split by an enzyme called Dicer, resulting in smaller RNAs. Dicer creates short double stranded RNAs that have 2 nucleotide long, 3 prime overhangs, which are called small interfering RNAs or siRNAs. Small interfering RNAs can form a ribonucleic protein

complex called RISC (or RNAi silencing complex). This complex includes Slicer, which is a protein which acts very much like a pair of scissors.

RISC first mediates the unwinding of the siRNA, a single stranded siRNA, that is coupled to RISC, then attaches to a target mRNA in a sequence specific way. The binding mediates target mRNA cleavage by a Slicer. The site of the splitting of the RNA falls in the middle of the region of siRNA, RISC bonded area, the cleaved mRNA can be recognised by the cell as being abnormal, and then subsequently destroyed. This whole process prevents translation from occurring, by silencing the expression of the gene, by destroying the mRNA, which would have been transcribed.

In plants the abnormal RNA that results from the RISC mediated splitting can also become a template for RNA dependant RNA polymerase (RDRP). This process relies on unprimed RNA synthesis, in which the abnormal RNA is used to help aid the process. The double stranded RNA is a substrate for Dicer activity, which helps to create more siRNAs, in some organisms which endogenous RNAi processes for example fungi, plants, worms and mammals, RNAi involves another step, to aid in the process. In this step, single stranded siRNAs, bind to their target mRNAs in a specific way. This serves as a primer for RNA dependant RNA polymerase to bond the RNA strand. Ribonucleic acid interference is very sensitive to natural sequence variation, the double stranded RNA molecule that is created, acts as a substrate for DICER, which splits it into siRNAs. This can unwind and prime RNA dependant RNA polymerisation, or together with RISC, can start to begin the splitting of target mRNAs. This coupled with RNAi spreading, is thought to underlie hereditary transmission of RNAi in worms and other organisms.

Discussion

It has been estimated that over 50% of the United Kingdoms dogs and cats are overweight. Being overweight or even obese has a detrimental health impact. Obesity can cause health problems like, type two diabetes and it can also put strains on joints, causing problems with walking and exercising in later life. In the future RNA interference could be able to solve the problem of obesity, before it has a huge impact on the dog's later life.

Recently, in a study carried out at the University of Liverpool, was an experiment to find out whether certain genes which code for adipokines were expressed in canine adipose tissues. Certain adipokines are linked to canine obesity. The experiment was carried out by using a technique called real-time polymerase chain reaction. This detects sequence-specific polymerase chain reactions products as they build up during the polymerase chain reaction amplification process. As the polymerase chain reaction product of interest is produced, real-time polymerase chain reaction can detect their accumulation and quantitatively identify the number of substrates present in the initial polymerase chain reaction mixture before the amplification process began.

There are a few variations of the real-time polymerase chain reaction, one of them is called a molecular beacon, which are short sections of single-stranded DNA .The sequence of each molecular beacon must be changed to detect the Polymerase chain reaction product of interest. When on a molecular beacon there are nine bases on one

end, they can pair with nine bases on the other end of the beacon. This complementary sequence allows the molecular beacon to form a structure in the shape of a hairpin. The loop section of the molecular beacon is composed of bases, these bases that are complementary to one strand of the polymerase chain reaction product that wants to be detected and quantified in the experiment.

Attached to opposite ends of the section molecular beacon with the 2 paired single stranded DNA, are a fluorescent reporter dye and a quencher dye. When the molecular beacon is in the hairpin shape, any fluorescence emitted by the reporter dye is absorbed by the quencher dye, therefore no fluorescence is detected.

Two polymerase chain reaction primers are then used to amplify a specific segment of DNA. As the polymerase chain reaction continues, the newly synthesized polymerase chain reaction products are denatured by high temperatures. As each strand of the product is separated, the molecular beacon also becomes denatured so therefore the hairpin structure is changed. As the temperature cools for the next set of primer annealing to take place, the molecular beacon forms base pairs with the appropriate strand of the polymerase chain reaction product. Any molecular beacons that do not fuse to the polymerase chain reaction product reform the hairpin-like structures and therefore are not able to fluoresce. However molecular beacons that fuse to the polymerase chain reaction product stop the ability of the quencher to absorb fluorescence from the reporter dye. So when it is excited by the appropriate wavelength of light, the reporter dye is able to fluoresce. Therefore, as polymerase chain reaction product accumulates, the amount of fluorescence is directly proportional to the amount of polymerase chain reaction product formed; therefore there is a linear increase in fluorescence.

Real-time polymerase chain reactions can be performed in a way so that more than one polymerase chain reaction product can be detected in a single reaction tube. For each sequence, there is a unique colour of fluorescent dye and therefore, each polymerase chain reaction product is associated with its own colour which is detected by the real-time polymerase chain reaction machine.

Using this method, mRNAs containing; adiponectin, leptin, angiotensinogen, plasminogen activator inhibitor-1, IL-6, haptoglobin, metallothionein-1 and 2, and nerve growth factor, of which, all of these substances found are adipokines. These substances were found in all adipose tissue examined. Whilst conducting this experiment TNF α was also found to be present in isolated adipocytes. These results demonstrated that white canine adipose tissue expressed major adipokines genes, which are linked to canine obesity.

Adipokines play a key role in regulating the metabolic rate and activity of the canine body. Adipokines also mediate inflammation, in promoting meal termination, regulating meal frequency and in insulin resistance. Therefore these hormones act to decrease the metabolic rate in canines which are already overweight; this additional decrease in metabolic rate is likely to lead to additional weight gain and causing the dog to have difficulty losing the extra weight.

Adipokines are also going to cause painful inflammation, for example in arthritis, due to the extra inflammation that the adipokines produce.

Regulation of diabetes mellitus may also prove to be more difficult in canines which adipose tissue which secretes adipokines. This is due to adipokines affecting the regulation of insulin resistance.

The number of chromosomes in *canis familiaris* is 38 paired chromosomes and a XX pair if a bitch, or XY pair if a dog. Adiponectin has an anti-inflammatory effect, unlike other adipokines, which helps the dog increase its metabolic rate, therefore increasing the rate of adipose tissue loss. Other adipokines have the opposite effect on the canine, for example leptin, which is located on chromosome 14, with the origin:

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1 tcagcaccca gggctgaggt ccagccgccg aagcatgtcc tggagggccg cctgcagtct
61 gctcagagcc accacctctg tggagtagag tgaggcttcc aggacgccgc ccaggctctc
121 aaaggtctcc aggccctgg cccggggcaa ggggcagctc ttggaggagg ccagcaggtg
181 gagaaggtcc cggaggttct ccaggtcatt agatatttgg accacatttc tggaatgcag
241 actgttgagg atctgttgg agatggccaa cgtctgttcc atcctggaca aactcaggac
301 tggttggagc ccaggaatga agtccagacc agcgaccctc tgtttggagg agacagactg
361 ctgggccaga ggaaaaggga gagcaggatc tcagacaagt cggttctgca aggtgtcggc
421 ctctcccatg agcgtggagg tccgctctct tgettgcctc ctctcctct tectcaccac
481 cctggcccag gcccttgttt cttgtgcccc aggggctgcc aagttccaa gctgtttctc
541 ggacttctgt actcctactc tccc aaatc acctgacct cccctccag accaatctc
601 gcaggtggtt ctctctgga aatggcctct gctgtagacc cagccatgag gectgattt
661 tacttagtga agtctcttcc ccaggcactg aggtctctct gaagtgcccc tcccagac
721 atcatcacc tctttgccc ccagaggatt cttgctccc agtcagggtc tccaaggct
781 cttacccct caggagtcaa ggetggacct ctcccacc cccctcccc cgccaaagag
841 tgtccatct cactctctg ctccagtaac actggttacc aggagtctc ccctctcca
901 ctgctgtgg cactcactct ctgcaccacc caagccggga cactctggac ctatcatgcg
961 tggcccatg aaagcaagga gactcataga cttctctt aaaatgccc aggtcttat
1021 ctagtctga gctgtccctg cttctggcca ctaacttct tctggctgc ccaaggccac
1081 tgtcagacat ccataatggg caggagatgt ggaatctct agattgtcc gtgctgggt
1141 ccgtgctggg tgagcaagca ggctttaag accaggggct actaaaacc acaactaac
1201 ttttaagag ggcagctgt gttctggtc ttttagggg ggtgagggga gatcacttg
1261 ttaactaac tgaatctct acaatcttg tgaagtcct tatgatagtc cactatcag
1321 gctatgaaa ctgaggctc aagacattga ggaattagct taagattta cagatagaga
1381 acagaagcgt gaagattcac atttggtct ctaattcca aatacagctg tcatctggga
1441 aatggctgaa ggtctaggcc tgtggacccc agggctaaca gtaacaggga gaagcttca
1501 ggggctagag tcatctagg aagaatgccc aatgatgagt acattctaga gatgtgacag
1561 aggctagag gggatgcaac taggagtctg gtcacctgcc agtgggacac aaagacctt
1621 atggctctct ttgctctag gcactaggac ttgcatcaga gaggaaggag agggagaagg
1681 tgggagatgg agagagagga agagaaaagg ctgctgtttg taccacgggg aggtgtcaag
1741 gccacatgg cccctatag tggctgcaca ggcctcaact accatctgag gtctgtgtt
1801 cccaagggc cggcttggg tctacttgg ctccagac cctcctacc gtgtgtgaaa
1861 tgtcattgat cctggcgaca atcgtcttga tgagggttt ggtgtcatcc tggactttc
1921 ggattggcag agcttcaaca caggacagat agggccaaag ccacaggaat cggcacagag
1981 gtccacaacg cat
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If leptin was not produced, then the dog's metabolism would be better, enabling the dog to lose weight easily, and stop excessively gaining weight. In white adipose tissue, when the Deoxyribonucleic acid was copied for chromosome 14, the section which codes for leptin, could be destroyed. By injecting double stranded RNA's (which can detect leptin) into white adipose tissue, the double stranded RNA's would

be delivered to white adipose tissue, by using cell-internalizing aptamers, which would deposit these double stranded RNA's only in these specific targeted cells.

These double stranded RNA's would then be recognised by a enzyme present in all "higher animal" cells, called Dicer, which then splits the double stranded RNA's into smaller RNA's, also called small interfering RNA's (siRNA's). Some of the small interfering RNA's then form a ribonucleic protein complex (RISC), which when forming this complex, would also creates the protein called SLICER within itself.

RISC then would initiate the unwinding of the siRNA's into 2 single strands. These single stranded siRNA's would then attach themselves to the origins of the mRNA, which was described earlier, in a sequence specific way. The sections which have been identified, will be cleaved, by Slicer. The cleaved sections of mRNA will be recognised by the cell as being abnormal, and therefore will be destroyed. This would therefore stop the expression of the leptin gene.

The process would then be repeated for the other adipokines which contribute to the inflammation. This would cause the only adipokine that is expressed in adipose tissue, to be adinopectin, which would increase the metabolic rate. Therefore this would enable the dog to lose the excess weight easily.

The process would only have to be done once, as the production of no other adipokines other than adinopectin only wants to happen while the dog is obese, or overweight. By only doing the gene interference once, the dog would have time to lose weight, but then start to produce the other hormones like leptin when a new set of mRNA is sent out to the ribosomes to manufacture hormones and proteins.

Surrounding the whole idea of gene interference in general are many ethical issues. Many religious groups oppose the idea of interfering with genes, because many of them would argue that the creator of everything on this planet, made everything in a specific way, for a specific reason, and therefore we shouldn't be silencing any genes.

Also gene interference would cost a lot to implement, so some people may argue that it isn't worth researching into RNA interference as many people wouldn't have the funding to back such procedures if they were to go ahead.

There are also issues surrounding "designer" pets. Many people would argue that stopping the expression of certain genes, would mean that people could erase imperfections of their pets, if there were no laws stating that certain procedures could not be implemented.

Conclusion

A current problem with RNA interference is to modify small interfering RNA to silence different genes, and once that is done, how to manufacture these small interfering RNA on a industrial scale to treat it in all animals of the same breed, and how to answer the problem of if a virus has been treated in an animal, viruses can mutate very quickly, so how would they keep up with the rate of mutation?

Although Richard Fire and Craig Mello's work in 1998 was surrounding RNA interference in worms, it would also work within different organisms, including zygotes. The possibilities of undesirable genes to be silenced are almost endless, and also could provide a solid base for stopping the transmission of harmful viruses, which cause death to many hundreds of animals each year.

With ethical backing, theoretically RNA interference could be used to find the function of certain genes, which would provide a great insight into treating diseases and diagnosis of conditions. The major concerns with RNA interference is that nobody currently really knows what underlying problems gene silencing could cause, whether gene silencing could be reversed and the problems surrounding "designer" animals.

With more being discovered about RNA interference in current research, we are getting closer to ultimately being able to control genetics, and therefore getting closer to being able to "play god".

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