OXFORD UNIVERSITY PRESS Toxicological Sciences

Are differences in microRNA regulation implicated in species-dependent response to toxicological exposures?

Journal:	*Toxicological Sciences*
Manuscript ID:	TOXSCI-12-0687.R1
Manuscript Type:	Review
Date Submitted by the Author:	04-Sep-2012
Complete List of Authors:	Koufaris, Costas; Imperial College London, Surgery & Cancer Gooderham, Nigel; Imperial College London, Molecular Toxicology
Key Words:	biomarkers < Safety Evaluation, exposure assessment < Risk Assessment, Gene Expression/Regulation
Society of Toxicology Specialty Section Subject Area:	Molecular Biology [120]

Are differences in microRNA regulation implicated in species-dependent response to toxicological exposures?

Costas Koufaris[1] and Nigel J. Gooderham[1]

[1]Biomolecular Medicine, Imperial College London, SW72AZ, UK

Contact information

Professor Nigel Gooderham, Department of Surgery & Cancer, Biomolecular Medicine, Imperial College London, Sir Alexander Fleming building, SW72AZ, UK

Tel: +44 (0)20 7594 3188

Email: n.gooderham@imperial.ac.uk

Abbreviations: miRNAs microRNAs, RISC RNA-induced silencing complex

Abstract

Equivalent exposures can result in qualitatively or quantitatively dissimilar toxic effects across species, with the underlying molecular mechanisms being often not well defined. In many cases, differences in metabolic handling of the chemical (metabolism and disposition) provide an explanation of these differences, but in other cases the explanation is less obvious. This variability in the outcome of toxicant exposures complicates the inter-species extrapolation of human hazard from animal testing data. MicroRNAs (miRNAs) are a family of non-coding RNAs that post-transcriptionally regulate the expression of their target genes that have fundamental roles in physiology, disease, and toxicological responses. Importantly, these non-coding genes are characterised by a high evolutionary flux, in terms of miRNA repertoire and functioning, even amongst closely related species. Furthermore, evidence is emerging that the enzymes of drug metabolism are also under miRNA regulation and thus offer a new twist to an old paradigm, whereby manipulation of the expression of these enzymes affect toxic outcomes. In this review we discuss how miRNA may contribute to the inter-species variability observed in the response to toxicant exposures. Although few studies have so far specifically examined the contribution of differences in miRNA regulation to species-dependent responses to toxicological exposures, we believe that this will be an area of intense research in the coming years.

In the past 10-15 years the discovery of endogenous, evolutionary conserved, non-coding RNAs with important physiological functions, has overturned the classic view of RNA as a passive intermediate of gene transcription. At present three main classes of non-coding, regulatory RNA are known in animals: the piwi-interacting RNAs (piRNAs), the short interfering RNAs (siRNAs), and miRNAs (Moazed,2009). Of these the most extensively studied are miRNAs that direct the RNA-induced silencing complex (RISC) to repress the expression of their target genes. MiRNA genes are located either within protein coding genes (30-40% of mammalian miRNAs) or within their own specified intergenic loci. The former are co-transcribed with their host genes, while the later have distinct promoters and regulatory elements (Carthew & Sontheimer, 2009). The product of the transcription is a long RNA transcipt which is subsequently cleaved in a multi-step process- involving among others the DICER and DROSHA enzymes- to generate the mature miRNA (Carthew & Sontheimer, 2009).

MiRNAs negatively regulate their gene targets by repressing mRNA translation and/or inducing mRNA degradation, although the precise mechanisms are still being investigated and debated (Eulalio *et al.*, 2008; Bartel, 2009; Carthew & Sontheimer, 2009; Filipowicz *et al.*, 2009). Bioinformatic analysis indicate that the majority of mammalian genes (~60%) are subject to miRNA regulation (Friedman *et al.*,2009). In addition, *in silico* and experimental data concur that a single miRNA can possibly regulate the levels of hundreds or thousands of genes (Grimson *et al.*,2007; Baek *et al.*,2008; Selbach et al,2008). As expected, given their prominent regulatory roles, miRNA expression is tightly regulated, being cell, tissue, and developmental stage dependent (Bartel, 2004). Nevertheless dysregulation of miRNAs have been linked to the initiation and development of numerous diseases including cancer, cardiovascular diseases, and central nervous system disorders (Taft *et al.*,2010). This review will focus on a different issue however, the possibility that miRNAs are a key factor in determining the differential response of species to toxic exposures.

Species-differences in toxicological responses

Animals are routinely used for the evaluation of potential human hazard associated with chemicals or other agents to which the public may be exposed. For example the carcinogenic potencies of regulated chemicals are evaluated using the rodent lifetime bioassay (Weisburger and Williams, 1981; Butterworth, 1995). The central concept behind the use of animals as laboratory experimental systems and for toxicity testing is the underlying

similarity in fundamental biology between humans and these animals. Animal testing is necessarily followed by an inter-species extrapolation in order to evaluate human risk. Nevertheless, fundamental differences in the intracellular regulatory machinery, biochemistry, and metabolism of humans and animals have accumulated due to natural selection or genetic drift, affecting toxicological responses and disease susceptibilities (Whysner & Williams, 1996; Cunningham, 2002; Kraunig *et al.*,2003; Rangarajan & Weinberg, 2003; Rangaragan *et al.*,2004; Holsapple *et al.*,2006; Lake, 2009). A classic example of such a species-difference in toxicological responses can be seen following exposure to d-limonene which induces nephropathy in male rats but not in humans, in a mechanism involving the a2μ-globulin protein (Whysner & Williams, 1996). The interaction of the chemical with the a2μ-globulin causes the accumulation of the protein in the kidney and subsequently cytotoxicity. In this specific case the mechanistic reason behind the species difference has been determined to be the male rat specific expression of a2μ-globulin (Whysner & Williams, 1996). However, in most cases the mechanistic bases for species-specific effects are less well defined. For example peroxisome proliferator and phenobarbital-type chemicals are also acknowledged to have distinct species effects, causing liver tumours by non-genotoxic mechanisms in rodents, but epidemiological data indicate that they are not harmful to humans (Klaunig *et al.*,2003; Holsapple *et al.*,2006; Lake, 2009). The susceptibility of rodents to liver tumours following treatment with these chemicals is associated with a rodent-specific induction of hepatic proliferation, although it is not clear why humans are resistant to hepatocarcinogenicity induced by these chemicals (Klaunig *et al.*,2003; Holsapple *et al.*,2006). In addition, there is probably another class of chemicals which may be falsely classified as human hazards based on animal data, when little or no epidemiological data are available. Lastly, some toxicants which are harmful to humans may not be detected by animal testing, due to human-specific effects.

Clearly a greater understanding of the cellular and molecular differences between species would be of great help in the interpretation of data collected from animal assays. In the post-genomic era there is considerable support for the notion that modifications in gene regulation is the primary force driving animal evolution, while the number and sequences of protein-coding genes are fairly consistent across animal genes (Levine & Tijan, 2003; Carol, 2005; Taft *et al.*,2007). Hence, it is probable that in most cases the molecular mechanisms associated with species-specific toxicological responses of animals are at the levels of gene regulation (e.g. change in gene expression patterns, regulatory elements in promoters and

enhancer, intracellular networks, epigenetic regulation, and non-coding RNAs). Intriguingly, fascinating evidence links altered miRNA regulation to the evolution of animal diversity and complexity. Evolution of animal miRNAs is a highly dynamic and ongoing process, even among closely related species (Berezikov, 2011), the rate of accumulation of new miRNAs during mammalian evolution was much higher than the respective for transcription factors (Chen & Rajewsky, 2007). MiRNAs are intrinsically highly evolvable molecules, as changes in a few nucleotides can have prominent functional effects (Chen & Rajewsky, 2007); and there is a correlation between the number of miRNAs and increased diversity and complexity in vertebrates (Hemberg, 2008).

Significantly, considerable evidence has also accumulated demonstrating the importance of miRNAs in the toxicological responses of organisms. We have recently reported that a wide range of chemical treatments affect liver miRNAs in a manner dependent on the time, dose, and mode of action of the chemicals (Koufaris *et al*., 2012). This is in agreement with the general view that miRNAs are responsive to cell stress (Reviewed in Lema & Cunningham, 2010). Dysregulation of miRNAs following toxic insults is expected to have prominent effects on the adaptive and adverse responses of animals, as these genes are the master regulators of important toxicological responses such as cell proliferation, apoptosis, and differentiation (Bartel *et al.*,2004) and also affect the activity of drug-metabolising and nuclear receptors (Yokoi & Nakajima, 2011). Therefore, besides being prominent regulators of the cellular phenotype, including the toxicological responses, we also know that miRNAs are evolutionary dynamic genes. Coupling these observations together suggests that differences at the miRNA level could be crucial in explaining the observed inter-species variability in the response to toxicant exposures. Here we will consider three distinct mechanisms by which miRNAs could in theory propagate such effects by altering the intracellular wiring of cells: differential regulation in response to toxic exposures, cross-species differences in miRNA:mRNA interactions, and the existence of lineage/species/strain specific miRNA repertoires.

Differences in miRNA regulation between species

Even though there is considerable evidence that miRNAs are affected by toxic exposures or cell stress (Lema & Cunningham, 2010), few studies have compared directly the effects of toxic treatments across species. Importantly these provide evidence that differential effects of the treatments on miRNA expression affect the outcome of the toxic exposure. Pogribny *et*

al., (2010) compared the effect of a methyl-deficient diet (which induced a condition in mice similar to non-alcoholic steatohepatitis) on the hepatic miRNAome of a sensitive (C57BL/6J) and a more resistant (DBA/2J) mice strain. They reported that between the two strains the effects of the treatment on miRNAs varied both qualitative (i.e. miRNAs only deregulated in one strain) and quantitative (much more pronounced changes in the sensitive strain). Importantly, the diffentially affected miRNAs were shown to be involved in the regulation of processes that are implicated in non-alcoholic steatohepatitis development e.g. lipid metabolism, apoptosis, and methylation (Pogribny *et al.*,2010). The authors of this study interpreted these results that the susceptibility of the two strains could be linked to their different miRNA profiles. A second intriguing study examined the effect of peroxisome proliferator treatment on the expression of mice liver miRNAs (Shah *et al.*,2007). Long-term treatment with such chemicals causes liver tumours in rodents *via* non-genotoxic mechanisms, involving an induction of hepatic proliferation, while there is no evidence of increased cancer risk in humans (Klaunig *et al.*,2003). Treatment with the peroxisome proliferator chemical Wy-14,643 resulted in the activation of a miRNA signalling cascade involving let-7c which drives hepatocellular proliferation (Shah *et al.*,2007). Intriguingly, activation of this miRNA cascade, and the associated hepatocellular proliferation, was not observed in humanised mice in which their wildtype receptor for peroxisome proliferator chemicals has been replaced by the human homolog (Shah *et al.*,2007). This study therefore suggests that differences in the regulation of a miRNA signalling cascade could be involved in the susceptibility of rodents to hepatocarcinogenicity following treatment with peroxisome proliferator chemicals.

Neither of the two studies mentioned above reporting differential effects of treatments on miRNAs of species/strains (Shah *et al*.,2007; Pogribny *et al*., 2010) had identified the mechanistic reason why the effects of the treatments on miRNA expression differed between the tested animals. At present much remains to be learned regarding the regulation of miRNA expression and biogenesis (Krol *et al.*2010). The regulatory circuits of miRNAs are highly complex, often being complicated negative or positive feedback loops with one or more transcription factors. Nevertheless, evidence supports that the transcription of miRNA genes is regulated in the same manner as protein-coding genes i.e. binding of transcription factors, DNA methylation, and histone modification being implicated, while regulation can also occur at the level of miRNA biogenesis (Krol *et al.*,2010). Importantly, plausible mechanisms by which miRNA regulation may differ between species can be envisioned e.g. changes in

regulatory or enhancer elements associated with miRNA promoters or in the processing of the precursor RNA after transcription, although these will have to be proven experimentally.

Difference in miRNA:mRNA interactions between species

Fundamentally the phenotypic effects of a miRNA are wholly determined by the set of proteins that they regulate. Given the widespread regulatory roles of miRNAs, the deregulation of a few or even a single miRNA:mRNA interactions could profoundly affect the phenotype of an organism. In support of this argument several human single nucleotide polymorphisms (SNPs) associated with a miRNA or miRNA binding site in target mRNA have been linked to increased susceptibility to diseases (Ryan *et al.*,2010). A cross-species shift in the targetome (miRNAs are predicted to have multiple targets) of a miRNA would therefore also alter the regulatory role of the miRNA. In animals the set of miRNA target genes are determined by appreciable, but not complete, miRNA-mRNA sequence complementarity which allows Watson-Crick pairing of the two nucleotide strands. Strong repression efficiency depends on the pairing of 7-8 nucleotide regions in the 3' UTR of mRNA to the "seed" region of the miRNAs (residues 2 to 8 on the 5' end) and a number of other factors such as the number of miRNA:mRNA pairing sites, supplemental pairing outside the seed region, position of miRNA:mRNA pairing sites, and the presence of AU rich nucleotide sequences (Grimson *et al.*2007; Bartel *et al.*,2009). MiRNA-mRNA pairings on 5'UTR or the coding regions of the mRNA can also induce post-translational silencing, but are less effective (Baek *et al.*, 2008, Grimson *et al.*, 2007). Since there is an optimal level for protein expression (i.e. both too high and too low expressions may be harmful to the organisms) it is postulated that there is a co-evolution of miRNA and their target mRNAs. In agreement with this it was found that miRNAs can have adverse effects on an organism's health when expressed in a different species (Tang *et al.*2010).

An altered targetome would mean that an observed miRNA deregulation following toxic exposure or cell stress may result in different phenotypic consequences in different species. In theory such a divergence in regulatory function of miRNAs between species could have occurred during evolution by (a) change in the mature miRNA sequence, especially the seed region (b) evolution of the mRNA target, especially the 3'UTR region (Berezikiv, 2011). Evolutionary divergence of the miRNA can occur by direct change in its sequence, altered processing during miRNA biogenesis or altered RNA editing. Alternatively, the mRNA can gain or lose miRNA regulation by a change in its 3'UTR sequence or by altering the length of

its 3'UTR. Importantly, changes in mature miRNA sequence would be expected to have significant cellular effects, as they would alter its targetome. Some proteins would no longer be subject to regulation by that miRNA while new miRNA-mRNA associations would occur. Indeed evidence shows that changes in the seed sequence of miRNAs occur mostly in lowly expressed miRNAs, presumably because these are more tolerated by organisms (Li & Liang, 2009). It would thus be expected that evolution of miRNA:mRNA regulation would most often progress by changes within the mRNA.

At present the best example of a disruption of mRNA:miRNA interaction having a significant phenotypic consequence is that of Texel sheep. In these animals, which are characterised by pronounced muscularity, it was found that a single change in the 3'UTR of myostatin creates a novel binding site for mir1 and miR-206 miRNAs which are highly expressed in muscle, resulting in the repression of myostatin and promotion of muscular hypertrophy by causing depletion of that protein (Clop *et al.*,2006). Another recent interesting study has reported that the progesterone receptor is subject to regulation by miR-96- a miRNA which we have shown to be induced in the rat liver after sustained treatment with several hepatotoxicants (Koufaris *et al*., 2012a; Koufaris *et al*., 2012b)- in primates but not in rodents, due to poorly conserved regions in the 3'UTR of the receptor (Liu *et al*.,2012). It is therefore certainly a possibility that evolutionary changes in miRNA:mRNA targeting could affect toxic responses and evidence is starting to emerge supporting this model.

Lineage/species/strain-specific miRNA repertoire

A fascinating discovery of the last five years has been the deep conservation of miRNAs throughout animal evolution (Grimson *et al.*,2008; Wheeler *et al.*,2009; Christodoulou *et al.*,2010). Once new miRNA genes are gained they are very stable and are rarely lost during evolution (Grimson *et al.*,2008; Wheeler *et al.*,2009). At the same time new miRNAs are continually being acquired by evolving species (Grimson *et al.*,2008; Wheeler *et al.*,2009). In fact, the rate of evolution of miRNAs is high enough that the miRNA repertoire differs even between closely related species and strains e.g. sister nematode species (de Wit *et al.*,2009), the human and chimpanzee brain (Berezikov *et al.*,2006), and even rat-strains (Linsen *et al.*2010). It appears that new miRNA genes can arise with such ease because they can emerge from a number of genomic sources, such as miRNA duplication, introns, pseudogenes, and transposable elements (Berezikov, 2011). Due to this ease of generation, the rate of

accumulation of new miRNAs during mammalian evolution was much higher than for transcription factors (Chen & Rajewsky, 2007).

At present it is not clear how many of the lineage-species specific miRNAs have functional roles. However, given the widespread effects that miRNAs can have, if even a small minority of the species-specific are functional, then the miRNA milieu could be intricately involved in the intracellular wiring of regulatory networks and, ultimately, affecting an organism's response to toxic insult. Importantly, one study has associated species-specific miRNAs to toxicological outcomes (Mor *et al.*,2011). This study found the activation of mouse-specific and rat-specific miRNAs in astrocytes (cells of the central nervous system that are important among other things in the blood-brain-barrier) following treatments with inflammation inducing LPS and IFN-γ and that these miRNAs regulate the TNF-α pathway. As more species-specific miRNAs are identified in the future it is expected that additional relationships with toxic outcomes will be uncovered.

Conclusion

Over millions of years of evolution species adapt to their specific environment and ecological niche. Animal diversity and complexity have been probably driven by changes at the level of gene regulation, with non-coding RNAs having prominent roles (Levine & Tijan, 2003; Carol, 2005; Taft *et al.*,2007). We are suggesting that species-differences in miRNA regulation, function, and repertoire can act to attenuate or amplify the toxic effects on an organism of exogenous exposures (**Fig.1**). This hypothesis is in agreement with both the dynamic nature of miRNAs during animal evolution and with their important toxicological functions. However, at present few studies have examined the contribution of miRNAs in the cross-animal variability of toxicological responses (Shah *et al.*,2007; Pogribny *et al.*,2010; Mor *et al.*,2011). Importantly, tools are already available that can begin to address this lack of knowledge: Deep-sequencing can be used to identify differences in miRNA repertoire of animals displaying variable toxic outcomes, followed by bioinformatics and experimental approaches to elicit the biological roles of these miRNAs; Global miRNA profiling platforms can characterise miRNAs displaying different expression patterns after chemical exposures; *in silico* tools can be used to identify evolutionary divergences in miRNA:mRNA interactions. In the future a deeper understanding of these miRNA related effects can potentially facilitate the inter-species extrapolation of animal testing, as well as the development of more suitable animal models.

Figure legends

Figure 1Mechanisms by which miRNAs could contribute to species-specific toxicological responses. On the top panel a species is shown in which treatment causes a toxic outcome, while in the lower panel a resistant species is shown (**A**) In the first species the compounds causes transcriptional induction of the conserved miRNA which subsequently promotes toxicity, while in the second species the compounds does not affect the expression of the miRNA; (**B**) The compound treatment induced the conserved miRNA in both species, but due to changes in miRNA sequence the miRNA no longer regulates the key protein ; (**C**) The compound causes toxicity only in the one species by affecting a species-specific miRNA.

References

Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P. & Bartel, D. P. (2008) The impact of microRNAs on protein output. *Nature,* 455 (7209), 64-71.

Bartel, D. P. (2004) MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell,* 116 (2), 281-297.

Bartel, D. P. (2009) MicroRNAs: Target Recognition and Regulatory Functions. *Cell,* 136 (2), 215-233.

Berezikov, E., Thuemmler, F., van Laake, L.W., Kondova, I., Bontrop, R., Cuppen, E., Plasterk, R.H. Diversity of microRNAs in human and chimpanzee brain. *Nature Genetics* 2006 38(12):1375-7.

Berezikov, E. Evolution of microRNA diversity and regulation in animals. *Nature Review Genetics* 2011 18;12(12):846-60

Butterworth, B. E., Conolly, R. B. & Morgan, K. T. (1995) A Strategy for Establishing Mode of Action of Chemical Carcinogens as a Guide for Approaches to Risk Assessments. *Cancer Letters,* 93 (1), 129-146.

Carroll, S.B. Evolution at two levels: on genes and form. *PLOS Biology* 2005 3(7).

Carthew, R. W. & Sontheimer, E. J. (2009) Origins and Mechanisms of miRNAs and siRNAs. *Cell,* 136 (4), 642-655.

Chen, K. and Rajewsky, N. The evolution of gene regulation by transcription factors and microRNAs. *Nature Reviews Genetics* 2007 8(2):93-103.

Christodoulou, F., Raible, F., Tomer, R., Simakov, O., Trachana, K., Klaus, S., Snyman, H., Hannon, G.J., Bork, P., Arendt, D. Ancient animal microRNAs and the evolution of tissue identity. *Nature* 2010 25;463(7284):1084-8.

Clop A, Marcq F, Takeda H, Pirottin D, Tordoir X, Bibé B, Bouix J, Caiment F, Elsen JM, Eychenne F, Larzul C, Laville E, Meish F, Milenkovic D, Tobin J, Charlier C, Georges M. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nature Genetics* 2006 ;38(7):813-8

Cunningham ML. A mouse is not a rat is not a human: species differences exist. *Toxicological Sciences* 2002 ;70(2):157-8.

de Wit E, Linsen SE, Cuppen E, Berezikov E. Repertoire and evolution of miRNA genes in four divergent nematode species. *Genome Research* 2009 ;19(11):2064-74.

Eulalio, A., Huntzinger, E. & Izaurralde, E. (2008) Getting to the root of miRNA-Mediated gene silencing. *Cell,* 132 (1), 9-14.

Filipowicz, W., Bhattacharyya, S. N. & Sonenberg, N. (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature Reviews Genetics,* 9 (2), 102-114.

Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research* 2009 19(1):92-105.

Grimson, A., Farh, K. K. H., Johnston, W. K., Garrett-Engele, P., Lim, L. P. & Bartel, D. P. (2007) MicroRNA targeting specificity in mammals: Determinants beyond seed pairing. *Molecular Cell,* 27 (1), 91-105.

Grimson A, Srivastava M, Fahey B, Woodcroft BJ, Chiang HR, King N, Degnan BM, Rokhsar DS, Bartel DP. Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature* 2008 30;455(7217):1193-7.

Heimberg AM, Sempere LF, Moy VN, Donoghue PC, Peterson KJ. MicroRNAs and the advent of vertebrate morphological complexity. *Proceeding National Academy Sciences* 2008 26;105(8):2946-50.

Holsapple, M. P., Pitot, H. C., Cohen, S. H., Boobis, A. R., Klaunig, J. E., Pastoor, T., Dellarco, V. L. & Dragan, Y. P. (2006) Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicological Sciences,* 89 (1), 51-56.

Klaunig, J. E., Babich, M. A., Baetcke, K. P., Cook, J. C., Corton, J. C., David, R. M., DeLuca, J. G., Lai, D. Y., McKee, R. H., Peters, J. M., Roberts, R. A. & Fenner-Crisp, P. A. (2003) PPAR alpha agonist-induced rodent tumors: Modes of action and human relevance. *Critical Reviews in Toxicology,* 33 (6), 655-780.

Koufaris, C., Wright, J., Currie, R.A., and Gooderham, N.J. (2012a) Hepatic microRNA profiles offer predictive and mechanistic insights after exposure to genotoxic and epigenetic hepatocarcinogens. *Tox. Sci.* doi: 10.1093/toxsci/kfs170.

Krol, J., Loedige, I., Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nature Review Genetics* 2010 ;11(9):597-610.

Lema, C. and Cunningham, M.J. MicroRNAs and their implications in toxicological research. *Toxicological Letters* 2010 5;198(2):100-5

Levine, M. and Tjian, R. Transcription regulation and animal diversity. Nature. 2003 10;424(6945):147-51.

Liang, H. and Li, W.H. Lowly expressed human microRNA genes evolve rapidly. Mol Biol Evol. 2009 ;26(6):1195-8.

Liu, J.L., Liang, X.H., Su, R.W., Lei, W., Jia, B., Feng, X.H., Li, Z.X., and Yang, Z.M. (2012) Combined analysis of microRNome and 3'-UTRome reveals a species-specific regulation of progesterone receptor expression in the endometrium of rhesus monkey. *J Biol Chem*. 20;287(17):13899-910.

Moazed, D. (2009) Small RNAs in transcriptional gene silencing and genome defence. *Nature,* 457 (7228), 413-420.

Mor, E., Cabilly, Y., Goldshmit, Y., Zalts, H., Modai, S., Edry, L., Elroy-Stein, O., Shomron, N. Species-specific microRNA roles elucidated following astrocyte activation. *Nucleic Acids Research* 2011 39(9):3710-23

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R. & Rajewsky, N. (2008) Widespread changes in protein synthesis induced by microRNAs. *Nature,* 455 (7209), 58-63.

Shah, Y. A., Morimura, K., Yang, Q., Tanabe, T., Takagi, M. & Gonzalez, F. J. (2007) Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation. *Molecular and Cellular Biology,* 27 (12), 4238-4247.

Pogribny, I.P., Starlard-Davenport, A., Tryndyak, V.P., Han, T., Ross, S.A., Rusyn, I., Beland, F.A. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Laboratory Investigations* 2010 ;90(10):1437-46.

Rangarajan, A. and Weinberg, R.A. Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice. *Nature Reviews Cancer* 2003 ;3(12):952-9.

Rangarajan, A., Hong, S.J., Gifford, A., Weinberg, R.A. Species- and cell type-specific requirements for cellular transformation. *Cancer Cell* 2004 ;6(2):171-83.

Ryan, B.M., Robles, A.I., Harris, C.C. Genetic variation in microRNA networks: the implications for cancer research. *Nature Reviews Cancer* 2010 ;10(6):389-402

Taft, R.J., Pheasant, M., Mattick, J.S. The relationship between non-protein-coding DNA and eukaryotic complexity. *Bioessays* 2007 ;29(3):288-99.

Taft, R.J., Pang, K.C., Mercer, T.R., Dinger, M., Mattick, J.S. Non-coding RNAs: regulators of disease. *Journal of Pathology* 2010 ;220(2):126-39.

Tang, T., Kumar, S., Shen, Y., Lu, J., Wu, M.L., Shi, S., Li, W.H., Wu, C.I. Adverse interactions between micro-RNAs and target genes from different species. *Proceedings National Academy Sciences* 2010 20;107(29):12935-40.

Weisburger, J. H. & Williams, G. M. (1981) Carcinogen Testing - Current Problems and New Approaches. *Science,* 214 (4519), 401-407.

Wheeler, B.M., Heimberg, A.M., Moy, V.N., Sperling, E.A., Holstein, T.W., Heber, S., Peterson, K.J. The deep evolution of metazoan microRNAs. *Evolution and development* 2009 ;11(1):50-68.

Whysner, J. & Williams, G. M. (1996) D-limonene mechanistic data and risk assessment: Absolute species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion. *Pharmacology & Therapeutics,* 71 (1-2), 127-136.

Yokoi, T. and Nakajima, M. Toxicological implications of modulation of gene expression by microRNAs. *Toxicological Sciences* 2011 ;123(1):1-14

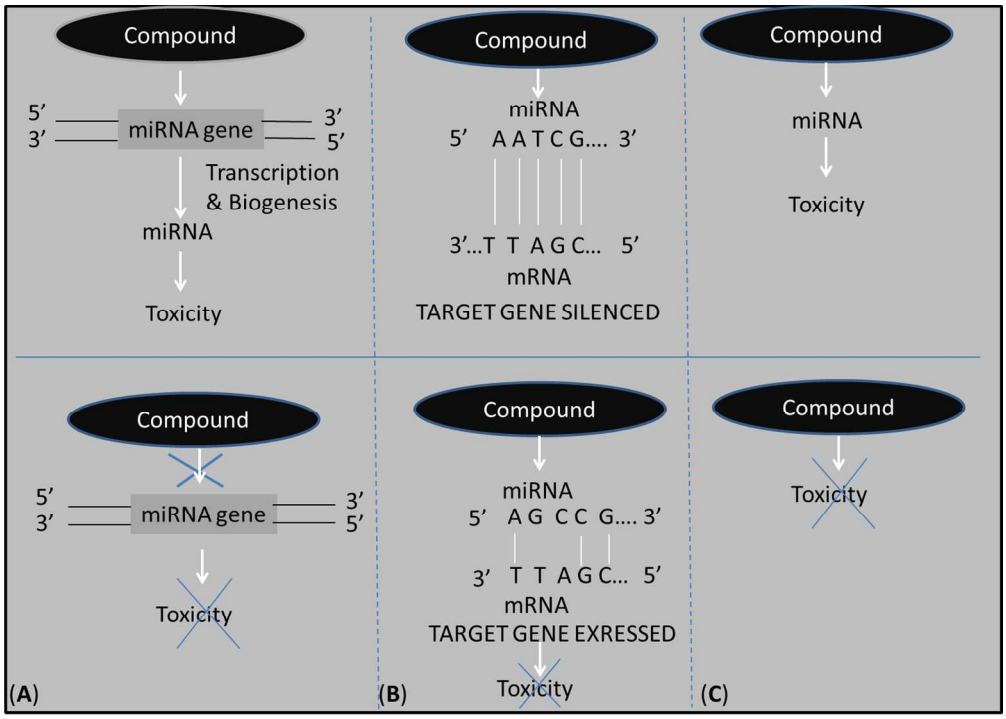

Figure 1. Mechanisms by which miRNAs could contribute to species-specific toxicological responses. On the top panel a species is shown in which treatment causes a toxic outcome, while in the lower panel a resistant species is shown (A) In the first species the compounds causes transcriptional induction of the conserved miRNA which subsequently promotes toxicity, while in the second species the compounds does not affect the expression of the miRNA; (B) The compound treatment induced the conserved miRNA in both species, but due to changes in miRNA sequence the miRNA no longer regulates the key protein ; (C) The compound causes toxicity only in the one species by affecting a species-specific miRNA.
176x125mm (300 x 300 DPI)