Development of an early biomarker assay panel to examine of carcinogenic and chemopreventive agents based on gene expression and microRNA expression changes

Doktori (Ph.D.) thesis

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1. INTRODUCTION

The fight against malignant tumors is a major health problem worldwide and a major obstacle to increasing life expectancy, affecting one out of five people in their lifetime and responsible for the death of one out of eight men or one out of eleven women. Current data show that the average five-year survival rate for cancer is less than 50%, despite the continuing progress in the therapies used, making the fight against cancer even more important in terms of prevention and early diagnosis. According to the World Health Organization (WHO) datas, about half of all malignant tumors are preventable and early diagnosis increases the chances of successful treatment. Consequently, the timely identification of harmful exogenic substances that may play a part in tumor development, along with the early detection of tumors are all important factors in the prevention and successful treatment of cancer.

1.1. Factors affecting the development of tumors

Environmental, endogenous and hereditary factors can contribute to the development of malignant tumors. In general, several environmental factors and also several genetic factors contribute to the development of the disease.

1.1.1. The role of hereditary factors

About 10% of tumor development is associated with inherited mutations, which may determine the rate of emergence of the tumour phenotype, the pathogenesis of the disease and the biological events subsequent to a potential recovery. However, the accumulation of mutations in key genes requires additional molecular events, such as spontaneous genetic defects that develop during cell proliferation and persist, or adverse effects of additional external environmental exposures.

1.1.2. Cell cycle disorder

Although the error rate of DNA replication is estimated to be less than one error per every billion bases copied, errors do occur. The resulting mutation may become fixed and passed on to the progenitor cells, leading to the accumulation of mutations, and ultimately to the development of tumors if genes involved in cell cycle regulation or DNA repair are damaged.

1.1.3. Environmental exposures

In most cases (80-90%), mutations are caused by adverse effects of external environmental factors, which usually induce DNA damage, i.e. they have mutagenic, genotoxic effects. The environment therefore plays a major role in the aetiology of tumors, irrespective of whether the environmental exposure is chemical, physical or biological.

Physical factors may include various kinds of ionising radiation, ultraviolet radiation, various dusts, fibres and crystals (e.g. asbestos), and even sedentary work, while biological factors usually include pathogens that promote carcinogenesis. The largest group of carcinogens belongs to the chemical carcinogens. Some chemical carcinogens induce mutations by binding directly to DNA in an unaltered form, but most of the chemical carcinogenic substances are indirect carcinogens. Examples include polycyclic aromatic hydrocarbons (PAHs) produced by incomplete combustion of organic materials (e.g. tobacco smoke, exhaust fumes, charcoal grilling, etc.).

7,12-dimethylbenz(a)anthracene (DMBA), used in our study, is a PAH commonly found in our environment. Cigarette smoke or exhaust fumes from cars are typical and significant sources of DMBA exposure. DMBA is a procarcinogen activated by CYP-metabolizing enzymes. The resulting metabolites can alkylate DNA or other cellular macromolecules, so DMBA may be involved in the initial stages of carcinogenesis, such as initiation and promotion. DMBA is also a genotoxic carcinogenic agent frequently used in cell culture and *in vivo* animal models, as it results tumors histologically similar to human tumors.

In summary, the genetic abnormalities developed at gene- or chromosome-level determine the biological properties of the damaged cells, their interaction with other cells, their invasive and/or metastatic potential and their response to the applied therapy, and thus its efficacy.

1.2. The role of molecular biomarkers in cancer prevention

Specific molecular biological events are potential early biomarkers of harmful environmental carcinogen exposures.

1.2.1. Study of mutations

In the early literature on biomarkers of tumorigenesis, studies of potential carcinogens have focused primarily on their carcinogenic effects, with studies based on genotoxicity and indicating mutagenicity at the chromosome or gene level. However, in order to prevent mutations and to have a deeper understanding of the mechanisms that initiate and promote carcinogenesis, it has become necessary to detect and to understand the early biological changes that precede mutation.

1.2.2. Study of gene expressions

In addition to the study of mutations and chromosomal aberrations, a more complex and comprehensive understanding of the tumor development process is possible by studying gene expression patterns as biomarkers. In most cases, differences in gene expression may also be observed when mutations arise, but at the gene expression level, effects that are independent of the mutations, may also be observed. Changes in gene expression may represent damage caused by a variety of chemical, physical or biological carcinogenic compounds, both in cell cultures and *in vivo* animal models. Furthermore, carcinogenic substances are not always genotoxic, yet they may have a significant effect on tumor formation by affecting the function of genes involved in carcinogenesis. A reduction in the expression of tumor suppressor genes that control the cell cycle may also be the result of an otherwise non-mutagenic epigenetic effect, such as DNA methylation. The final result in this case is also a reduction in the amount of the normally functioning tumor suppressor protein, a reduction in its effect. Therefore, a change in gene expression does not necessarily require the mutation of that particular gene.

1.2.3. Study of microRNA expression

In addition to understanding the diverse gene expression patterns, mapping the effects of microRNAs (miRNAs) involved in gene regulation provides opportunities to understand further details.

1.2.3.1. The miRNAs

The miRNAs are specialised highly conserved RNA molecules of 19 to 22 nucleotides in length found in plants, animals, fungi and some viruses, which, via their binding to the corresponding 3' UTR (three prime untranslated region) of partially or fully complementary messenger RNAs (mRNAs), are able to affect their translation and through this the protein synthesis itself.

1.2.3.2. The role of miRNAs in gene regulation

The miRNAs regulate approximately 30%-50% of human gene expressions, thereby influencing important cellular biological processes related to tumor formation, such as cell cycle, cell differentiation or apoptosis. The regulatory effects can be very diverse, as a single miRNA can affect multiple target genes and the biogenesis of miRNAs is influenced by other factors, as well. Depending on the nature of the genes they regulate, miRNAs can also exert either onco- or tumor suppressor effects. The complexity of regulation is illustrated by the fact that some miRNAs can exert both onco- and tumor suppressor effects, since a single miRNA can be partially or fully complementary to several mRNAs. The opposing effects may be tumor-specific, but dose-dependent differences are also possible.

1.2.3.3. The miRNAs as biomarkers

It can be stated, that many of the miRNAs are present in fragile genomic regions and they mostly display altered expression pattern in malignantly transformed samples. However, evidence based on experimental studies and on analysis of clinical histopathological samples suggests that specifically expressed miRNAs can be observed for all carcinogenic effects and, because miRNAs are stable in serum, they have the potential to serve as early epigenetic biomarkers for the detection of various cancers and other diseases.

1.2.4. miRNAs and genes included in the study

The miRNAs examined (miR-9-1, miR-9-3, miR-29a, miR-124-1, miR-132, miR-134, miR-155, miR-212, miR-330) previously have been reported to show altered expression in many malignant tumors and via various signalling pathways are involved in the

regulation of oncogenes or tumour suppressor genes, in the growth, proliferation, invasion, migration, metastasis or apoptosis of cells. The study of the interactions of these miRNAs and of the mTORC1 gene with DMBA may provide important insights into the mechanisms underlying the pathogenesis of tumors induced by chemical carcinogens. Through understanding our results, we can further explore the putative roles of the examined miRNAs and mTORC1 in tumorigenesis and evaluate their potential application as early biomarkers in the development of an assay panel.

1.3. Aims

Due to the nature of cancer, prevention is of increasing importance in the fight against the development of the disease, where it is necessary to detect molecular biological phenomena indicating effects/damages caused by carcinogenic substances as early as possible, well before the onset of clinical symptoms.

Consistent with this, the aim of our study is:

1. to evaluate the altered expression patterns of the the selected nine miRNAs (miR-9-1, miR-9-3, miR-29a, miR-124-1, miR-132, miR-134, miR-155, miR-212, miR-330) and of the mTORC1 gene as early biomarkers for the detection of DMBA-induced carcinogenic effects 24 h after the DMBA treatment.

2. to explore the interactions of the mTORC1 gene and of the miRNAs examined.

3. based on the results, to use the miRNAs and the mTORC1 gene to develop an assay panel that can either be suitable for the identification of early signs of carcinogenic processes or, using a combination of proven carcinogenic and putatively chemopreventive agents, for the rapid screening of anticarcinogenic substances.

2. MATERIALS AND METHODS

Two groups of CBA/Ca mice were used in our study. Both control and DMBA-treated groups consisted of 12 (six males and six females) 6-8 weeks old mice. Both the treated and the control groups received 0.1 mL of corn oil (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally, but in the treated group, 20 mg/bwkg DMBA was dissolved in the

corn oil. After 24 h of DMBA exposure, the mice were euthanized, cervical dislocation was performed, and the livers, kidneys, and spleens were removed. Total cellular RNA level was determined as described below. In accordance with the guidelines concerning laboratory animals, mice received humane care. The experiment was approved by Regional Animal Ethical Committee Pécs and conducted according to the current ethical regulations (ethical permission no.: BA02/2000-79/2017).

2.1. Isolation of Total RNA

Total cellular RNA was isolated using TRIZOL reagent, according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). The RNA quality was determined by NanoDrop absorption photometry. Only RNA fractions with A > 2.0 at 260/280 nm were used for the reverse transcription polymerase chain reaction process.

2.2. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

the one-step PCR, including reverse transcription and target amplification, was performed in a 96-well plate using Kapa SYBR FAST One-step RTQCR kit (Kapa Biosystems, Wilmington, MA, USA) on a LightCycler 480 qPCR platform. Primers of the mTORC1 gene, the examined miRNAs (miR-9-1, miR-9-3, miR-29a, miR-124-1, miR-132, miR-134, miR-155, miR-212, miR-330) and the internal control gene (mouse U6), were synthetized by Integrated DNA Technologies (Integrated DNA Technologies Inc., Coralville, IA, USA). The sequences were taken from previous publications.

2.3. Calculations and Statistical Analysis

Relative miRNA expression levels were calculated and compared using the $2^{-\Delta\Delta CT}$ method. During the statistical analysis for the testing the distribution of results, we used the Kolmogorov–Smirnov test. To compare means, we used the Levene's type T-probe. For calculations and analysis, IBM SPSS 21 (International Business Machines Corporation, Armonk, NY, USA) statistical software was used. We determined the level of statistical significance at $p < 0.05$.

3. RESULTS

For both the negative control and the treated group, we determined the expression of the miRNAs and the mTORC1 gene 24 hours after DMBA treatment. When processing the results, not only the average results of the treated and control groups, but also the sex and organ-specific lesions are summarized in order to understand even the deeper correlations between the interactions of miRNA and gene expression changes induced by DMBA exposure. Compared to the control group, the differences were expressed as percentages, where the untreated control was considered to be 100% (Figure 1-8). In summary, based on the results of the treated group, it can be stated that DMBA treatment after 24 h has an effect on the expression of the miRNAs examined and mTORC1 compared to the control group, however, the magnitude and direction of the effect is specific according to the gene, miRNA, tissue and/or sex examined.

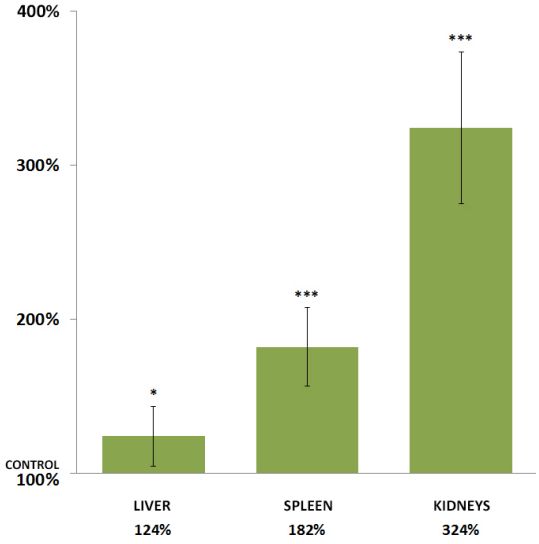

Figure 1: Changes in the expression of mTORC1 gene 24 hours after DMBA treatment in the liver, spleen and kidneys of female CBA/Ca mice (*p<0.05; ***p<0.001).

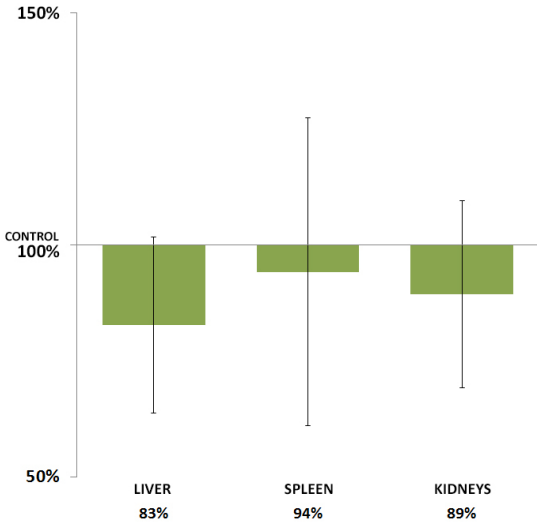

Figure 2: Changes in the expression of mTORC1 gene 24 hours after DMBA treatment in the liver, spleen and kidneys of male CBA/Ca mice.

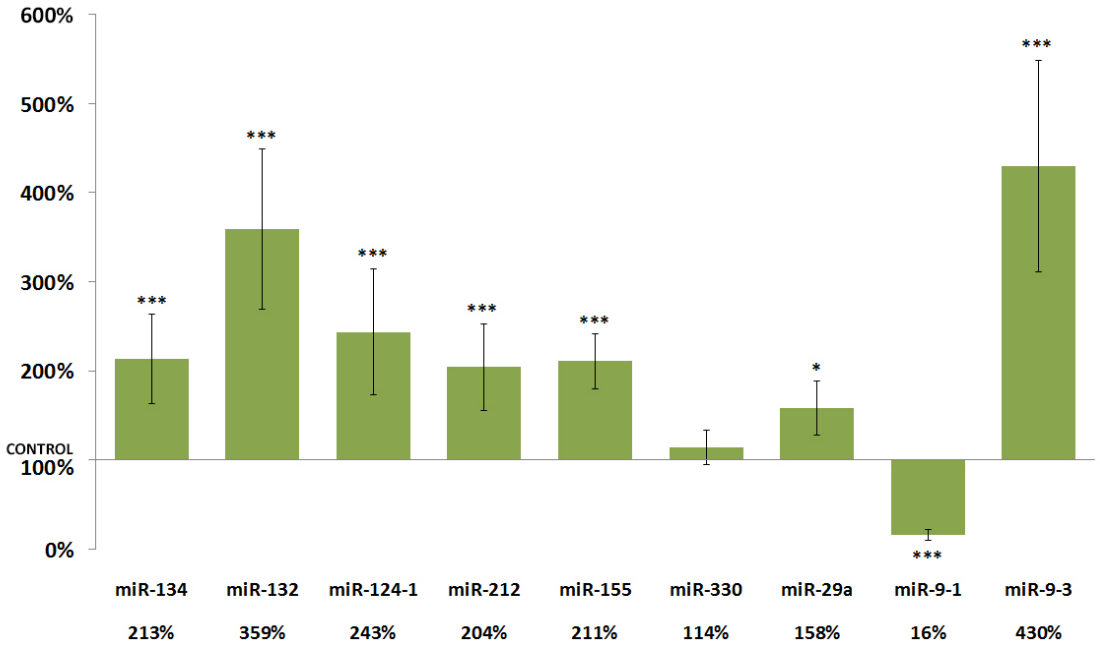

Figure 3: Changes in the expressions of miRNAs examined (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330), 24 hours after DMBA treatment in the liver of female CBA/Ca mice (*p<0.05; ***p<0.001).

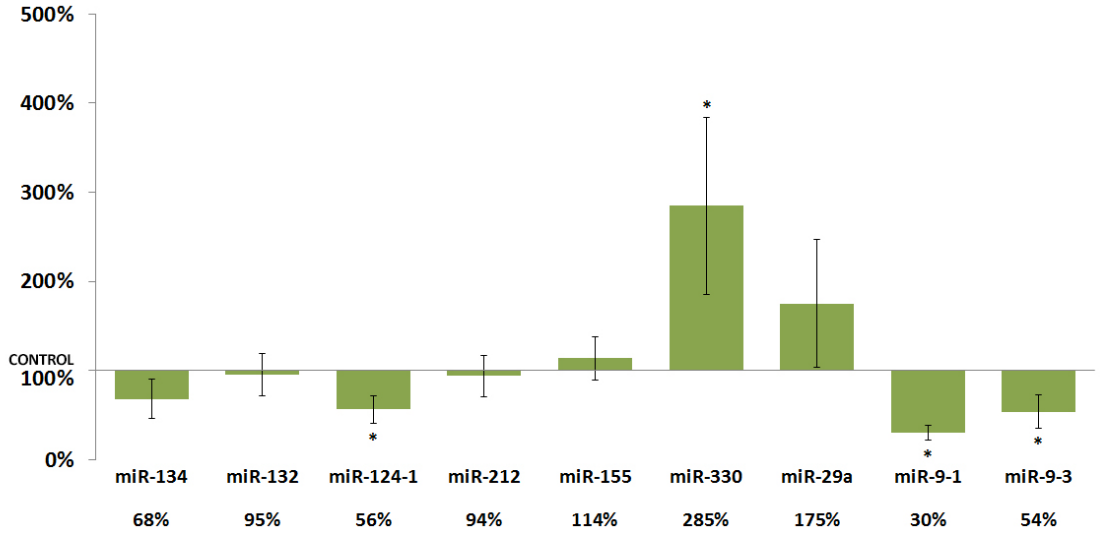

Figure 4: Changes in the expressions of the miRNAs examined (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330), 24 hours after DMBA treatment in the liver of male CBA/Ca mice (*p<0.05).

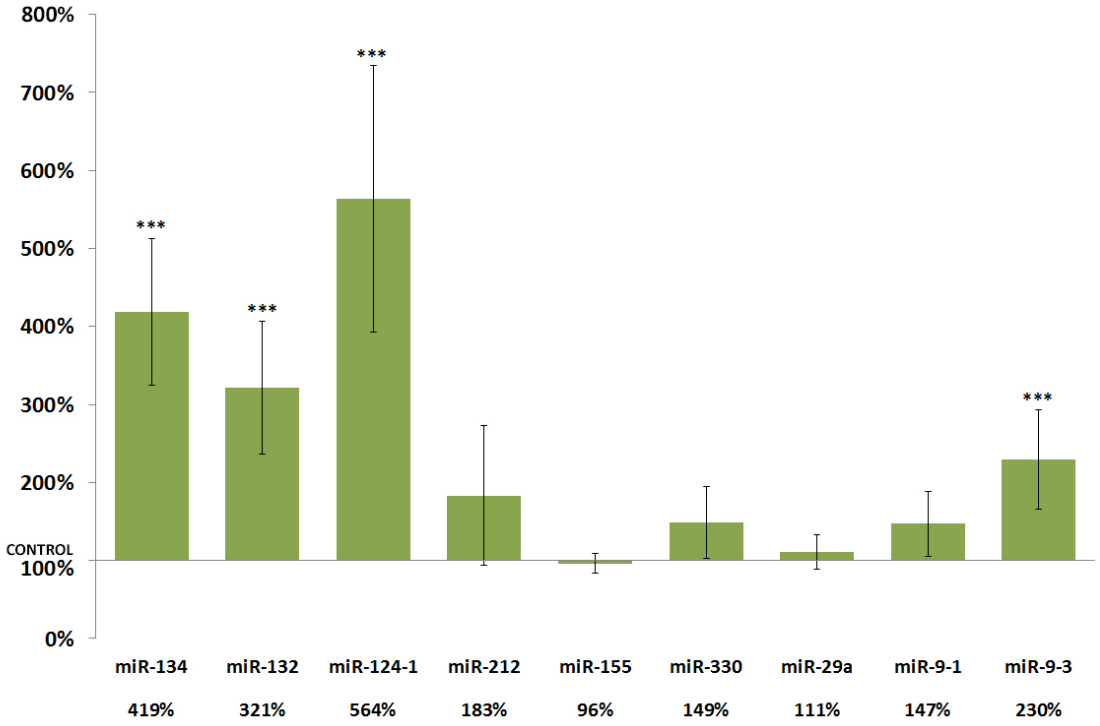

Figure 5: Changes in the expressions of the miRNAs examined (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330), in the spleen of female CBA/Ca mice 24 hours after DMBA treatment (*p<0.001).**

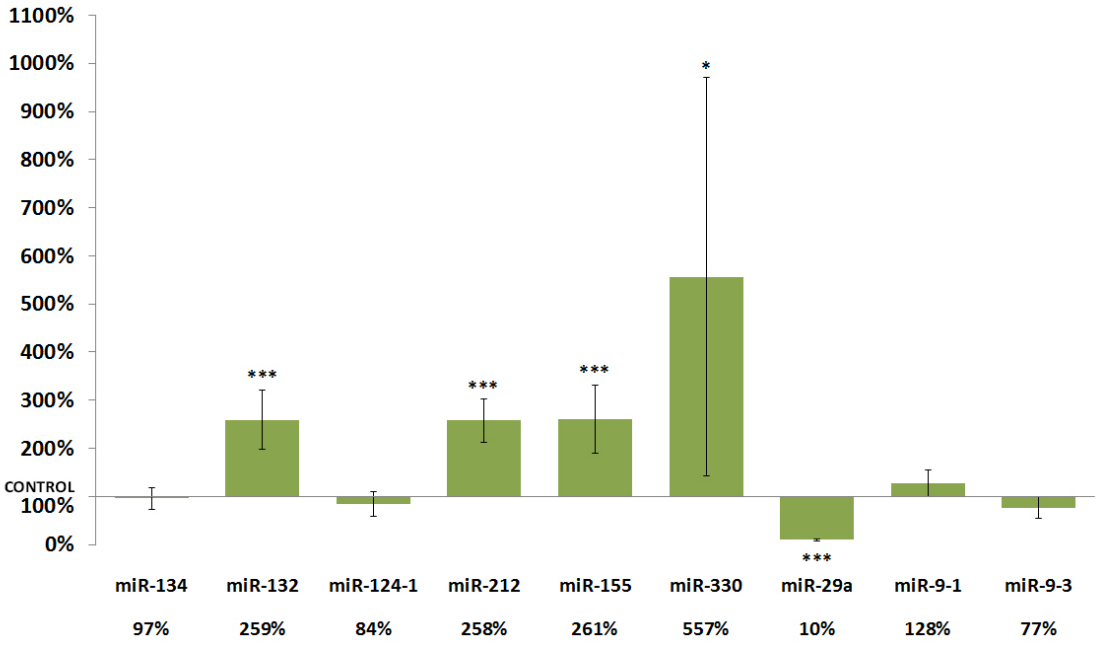

Figure 6: Changes in the expressions of the miRNAs examined (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330), in the spleen of male CBA/Ca mice 24 hours after DMBA treatment (*p<0.05; ***p<0.001).

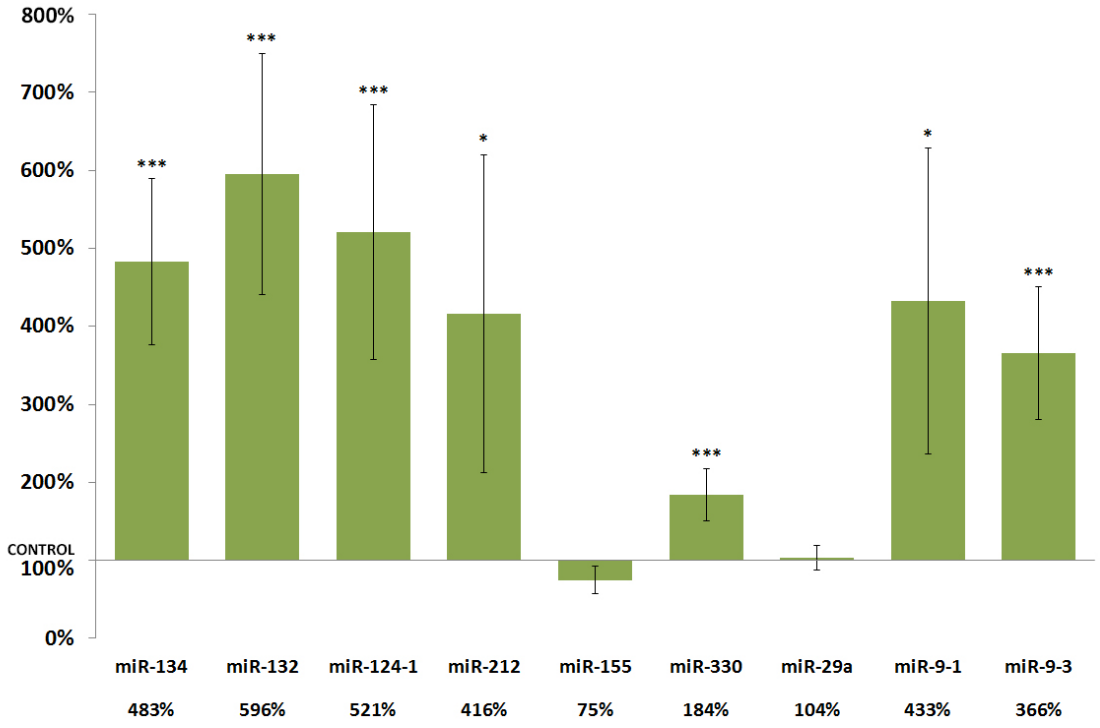

Figure 7: Changes in the expressions of miRNAs (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330) examined, 24 hours after DMBA treatment in kidneys of female CBA/Ca mice (*p<0.05; ***p<0.001).

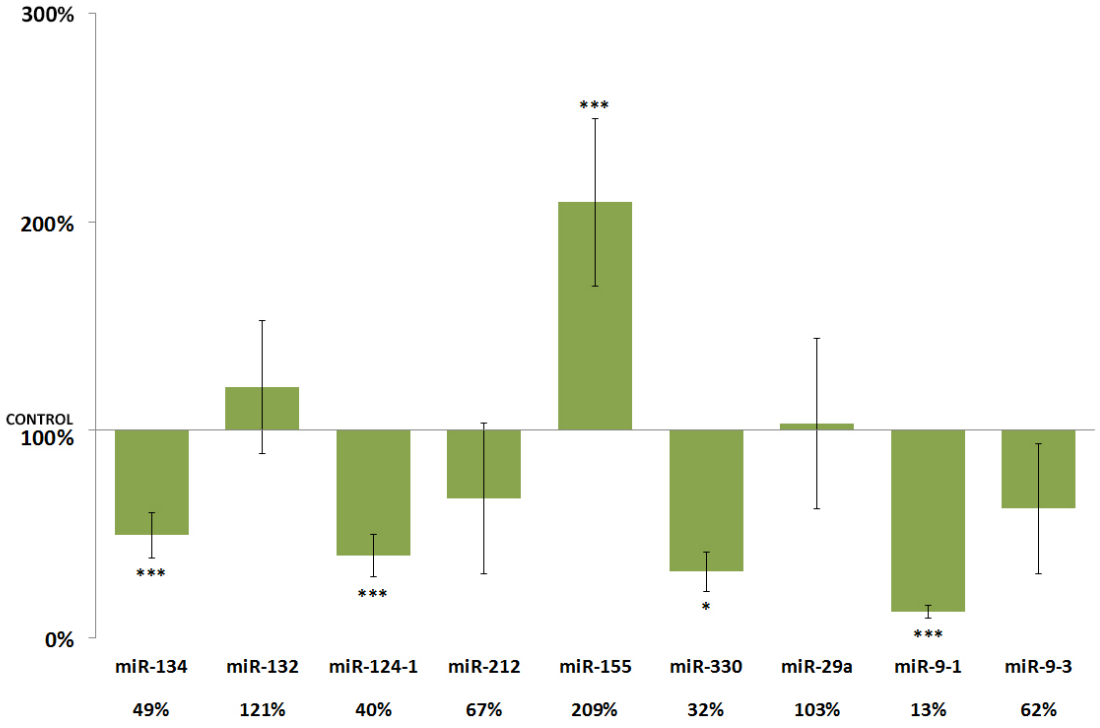

Figure 8: Changes in the expressions of miRNAs (miR 9 1, miR 9 3, miR 29a, miR 124 1, miR 132, miR 134, miR 155, miR 212 and miR 330) examined, in the kidneys of male CBA/Ca mice 24 hours after DMBA treatment (*p<0.05; ***p<0.001).

4. DISCUSSION

4.1. mTORC1

The activation of mTOR signalling induces several oncogenic processes, including the promotion of the growth, survival and proliferation of cancerous cells. mTORC1 functions as a mediator in the oncogenic effects of DMBA-induced TGF-α (transforming growth factor alpha) and IGF-1 (insulin-like growth factor 1). In addition, it potentiates the proliferation-promoting effect of IGF-2 (Insulin-like growth factor 2) in combination with DMBA. Its potential oncogenic role in females was also demonstrated in the present study model, as a significant increase in mTORC1 expression was observed in all organs of female animals examined 24 hours after DMBA exposure. In male mice, however, we still observed decreasing results, but these were not significant. This may be due to the fact that the tumor suppressor gene PTEN (phosphatase and tensin homolog), which is highly expressed in the absence of testosterone, is an inhibitor of mTORC1. Therefore, we found the expression of the mTORC1 gene in females in the model used in the present study to be applicable for testing carcinogens.

4.2. miR-132

It is known that miR-132 regulates the proliferation, apoptosis, migration and invasion of liver tumor cells by repressing the transcription factor SOX (SRY-related HMG-box 4) induced by DMBA. Furthermore, DMBA is known to increase TGF-β1 levels in a dose-dependent manner, and increased expression of TGF-β1 increases the expression of miR-132. However, miR-132 blocks DMBA-activated TGF-β1/Smad2/3 signalling. This suggests that there is a mutual feedback between TGF-β1 and miR-132, with DMBA having an enhancing effect and with miR-132 having a silencing effect on it. Previous reports has also indicated that miR-132 suppresses the Akt/mTOR signalling pathway, which is activated by DMBA and involved in promoting tumor formation, and this interaction was reflected in our results, as well. We obtained a similar expression pattern of miR-132 and mTORC1, suggesting that miR-132 expression may have increased in response to an increase in mTORC1 expression. In males, the change was significant only in the spleen, whereas in females we observed significant and highly

statistically significant increases in all the organs examined, correlating with literature data, thus miR-132 can be used as a biomarker with sex specificity in female mice.

4.3. miR-212

The underlying mechanism of the diverse results observed for miR-212 may be, that it can exert both tumor suppressor and oncogenic effects, for example by regulating the FOXA1 (Forkhead Box A1) gene, an effect that is non-specific, as FOXA1 is indispensable for both estrogen and androgen signalling The increase in expression in females by repressing FOXA1 reduces the protective effect of estrogen, while in males the reduced miR-212 level promotes the oncogenic effect observed in androgen signalling. Despite the significant and robust increase in miR-212 expression in the liver and kidneys of females and in the spleen of males, based on the overall results obtained (which include all organs examined in both sexes), the use of miR-212 as a biomarker was discarded in the present study due to lack of significance.

4.4. miR-124-1

The tumor suppressor effect of miR-124-1 has been observed through several mechanisms. The direct target of miR-124-1 is the CASC3 protein, through which it is able to inactivate the p38 MAPK, JNK or ERK signalling pathways, thereby inhibiting cell proliferation. In addition, DNA damage-induced nuclear factor-kappa B (NF-κB) activation is a major obstacle to effective antitumor therapy, but miR 124-1 represses the expression of TRAF6 (TNF Receptor Associated Factor 6), thereby inhibiting the NF-κB signalling pathway. As the expected increase in the expression following carcinogen exposure was only observed in the female group, where a highly significant and significant increase was observed in all the three organs examined, miR 124-1 was found to be applicable as a biomarker in females only. However, in males, DMBA treatment caused a decrease in miR 124-1 levels, but the decrease was not significant in all organs. DMBA activates the transcription factor STAT3 (signal transducer and activator of transcription 3), which has a repressive effect against miR-124, and a STAT3-inducing effect was also observed for testosterone, which could coherently explain the downregulation of miR 124-1 in the male group by DMBA/ which with DMBA may coherently explain the downregulation of miR-124-1 in the male group.

4.5. miR-155

For miR-155, a unidirectional positive change was observed in both sexes only for the liver among all the organs examined in both sexes, although although the changes were not significant inmales, which may be explained by the stimulatory effect of estrogens on miR-155 expression. In addition, the metabolic activation of DMBA is carried out by CYP enzymes, mainly cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1), which are typically synthesized in high amounts in the liver, and thus the effect of DMBA on miR-155 expression may be more rapid and dominant in this organ. It has also been observed that miR-155 expression is elevated in liver injury and exerts a protective effect by suppressing NF-κB signalling and by reducing inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) or interleukin 6 (IL-6). However, miR-155 has also been identified as an oncomiRNA in different types of human tumors, which, via its increased expression inhibits the activity of tumor suppressor targets such as TP53INP1 (tumor protein p53 induced nuclear protein 1), RhoA (Ras homolog family member A) GTP binding protein, or VHL (von Hippel Lindau) tumor suppressor protein. Based on the varied data in the literature and in our study, miR-155 can not be used as an early biomarker in our target assay panel.

4.6. miR-330

As a result of DMBA treatment, a smaller increase in the expression of miR-330 tumor suppressor was observed in the spleen and liver of female mice than in male mice. This may be due to the previously mentioned high expression of the proapoptotic PTEN tumor suppressor gene in the absence of testosterone, the gene product of which reduces the oncogenic effect of c-Myc proteins which are highly expressed in response to the effect of DMBA treatment. The increase in the expression of the tumor suppressor miR-330 measured in the kidneys of female mice is consistent with the observation that it reduces estrogen-induced risk increase for RCC. Based on our results and literature data, we can conclude that the expression levels of miR-330 could be used as a biomarker mainly in male animals, but the results were not identical in terms of their direction in all the organs examined (an important criterion for selection), and its use as a biomarker was therefore discarded.

4.7. miR-29a

An increase in c-Myc gene expression was observed 12 hours after DMBA exposure. However, the Myc proto-oncogene represses miR-29a expression in, for example, pancreatic cancer cells, resulting in a downregulation of LOXL2 (Lysyl Oxidase Like 2), thereby promoting tumor formation. Namely, LOXL2 is involved in tumor cell invasion, metastasis, angiogenesis and malignant transformation. Thus, miR-29a has an impact on the tumorigenic potential of DMBA through the Myc/miR-29a/LOXL2 pathway. According to literature data miR-29a is involved in a number of cell biological processes that have not yet been fully elucidated. Considering that a significant decrease in miR-29a expression was observed only in the spleen of male animals among the organs we examined, and that the regulation of this mechanism is complex, apparently controversial and is still unexplored, miR-29a expression as a biomarker is not an option in the animal model we used.

4.8. miR-9-1 és miR-9-3

Literature shows that miR-9 inhibits the progression of HCC as a tumor suppressor, and that the expression of miR-9-1 significantly decreased in HCC tissues and negatively correlated with overall survival of HCC. It is known, that silencing of miR-9-1 enhances the expression of oncogenes which is induced by the RUNX1 RUNX1T1 transcription factor complex that it regulates. Furthermore, miR-9 downregulation has been shown to serve as an early biomarker for the development of various malignancies, such as breast cancer. The oncoproteins c-Myc and n-Myc acting at the miR-9-3 locus (with increased expression in response to DMBA) induce an increase in the expression of miR-9 in tumor cells, which (this time acting in an oncogene supporting manner), via amplification of E cadherin, causes a further increase in the expression of c-Myc and, through this, promotes hepatocellular carcinoma formation. This finding is supported by the fact that, we observed a significant difference between miR-9-1 and miR-9-3 expression in the liver of female mice.

In the female group, despite the protective estrogen, expression of miR-9-3 increased 24 h after DMBA treatment in all the organs examined compared to miR-9-1, where a decrease was observed in the liver. As could be expected on the basis literary data,

according to our own study, miR-9-3 miR-9-3 can be well used as a biomarker in the case of female mice, as well. Thus, by studying miR-9-3, and extending the experimental design by complementing DMBA treatment with presumably chemopreventive agents, new molecular epidemiological associations are likely to be discovered. However, the use of changes in expressions generally observed for miR-9-1 and found for miR-9-3 in males, based on the present results was discarded due to lack of significance.

4.9. miR-134

The strong expression of miR-134 leads to reduced expression levels of c-Myc oncogene and cell cycle regulatory proteins Cyclin-E and Cyclin-D1, and also exerts tumor suppressor effects by regulating intracellular signalling pathways, such as the RAS/MAPK/ERK pathway or RAS/PI3K/AKT signalling that influence cell proliferation, invasion and apoptosis. The underlying factors for the clear and significant sex differences observed in our results may be the upstream regulators of miR-134 such as the transcription factor NF κB. DMBA increases the expression of NF-κB, which is also an inhibitor of miR-134, among others, so DMBA may decrease the expression of miR-134 through a stronger expression of NF-κB, which may explain the decreases observed in male mice.

In female mice, however, the estrogen receptor (ER) inhibits NF-κB activation, thus blocking its repressive effect on miR-134, which in this case could lead to a marked increase in expression. Finally, taking into account the effect of sex-specific hormonal differences and the statistically significant marked increases in the organs examined, miR-134 expression was found to be applicable as a biomarker only in females.

5. CONCLUSIONS

In summary, for the mTORC1 gene and for the miRNAs examined (miR-9-1, miR-9-3, miR-29a, miR-124-1, miR-132, miR-134, miR-155, miR-212, miR-330), we observed consistent and substantial, statistically significant changes showing in the same direction in all of the organs examined, for miR-134, miR-132, miR-124-1, miR-9-3 miRNAs of female mice and for the mTORC1 gene. These essential test criteria were found to be less fulfilled for the other miRNAs or for the examined organs of the male animals, in general. Therefore, in line with my objectives, the development of the assay panel designed in our study was achieved by including these miRNAs as well and mTORC1 gene.

Our results highlight that while gene expression and miRNA expression changes are sensitive biomarkers, they can be affected by potential confounders. Thus, the development of an early carcinogenicity test system requires careful selection of the genes and miRNAs used, as even sex-specific hormonal differences can significantly affect expression patterns. Moreover, miRNAs and genes influence each other's expression/effects directly or sometimes indirectly through the involvement of other gene products, which could be observed in several aspects for the gene and miRNAs in our study.

The complexity of the molecular processes involved in carcinogenesis makes it necessary to extend the test systems to the widest possible range of signaling pathways and their regulators. The miR-9-3, miR-124-1, miR-132, and miR-134 miRNAs and the mTORC1 gene are involved in signaling pathways such as RAS/MAPK/ERK, RAS/PI3K/AKT, p38 MAPK, or JNK, which typically show higher activity in the early phase of carcinogenesis.

Applied in practice, the five biomarker-based assay panel designed to predict carcinogenesis could provide a valuable tool for further investigation of chemopreventive and/or complementary therapeutic tumor suppressor compounds, thus opening new opportunities for reducing tumor incidence and mortality by using them as a tool for primary, secondary, and tertiary prevention.

6. NEW RESULTS

Our research has confirmed the following new results:

1. The mTORC1 gene expression shows a statistically significant increase in the liver*, spleen*** and kidneys*** of female CBA/Ca mice 24 hours after DMBA exposure (*p<0.05; ***p<0.001).

2. The expression of miR-9-3 miRNA shows a statistically significant increase in the liver***, spleen*** and kidneys*** of female CBA/Ca mice 24 hours after DMBA exposure (***p<0.001).

3. The expression of miR-124-1 miRNA shows a statistically significant increase in the liver***, spleen*** and kidneys*** of female CBA/Ca mice 24 hours after DMBA exposure (***p<0.001).

4. The expression of miR-132 miRNA shows a statistically significant increase in the liver***, spleen*** and kidneys*** of female CBA/Ca mice 24 hours after DMBA exposure (***p<0.001).

5. The expression of miR-134 miRNA expression shows a statistically significant increase in the liver***, spleen*** and kidneys*** of female CBA/Ca mice 24 hours after DMBA exposure (***p<0.001).

6. Increases in the expression of miR-9-3, miR-124-1, miR-132, and miR-134 miRNAs and mTORC1 genes are early biomarkers of DMBA-induced carcinogenicity in the liver, spleen and kidneys of female CBA/Ca mice 24 hours after exposure.

PUBLICATIONS

Publications on which the dissertation is based

Tomesz A, Szabo L, Molnar R, Deutsch A, Darago R, Raposa BL, Ghodratollah N, Varjas T, Nemeth B, Orsos Z, Pozsgai E, Szentpeteri JL, Budan F, Kiss I. Changes in miR-124-1, miR-212, miR-132, miR-134, and miR-155 Expression Patterns after 7,12-Dimethylbenz(a)anthracene Treatment in CBA/Ca Mice. Cells. 2022 Mar 17;11(6):1020.

IF: 7.666

Tomesz A, Szabo L, Molnar R, Deutsch A, Darago R, Mathe D, Budan F, Ghodratollah N, Varjas T, Nemeth B, Kiss I. Effect of 7,12-Dimethylbenz(α)anthracene on the Expression of miR-330, miR-29a, miR-9-1, miR-9-3 and the mTORC1 Gene in CBA/Ca Mice. In Vivo. 2020 Sep-Oct;34(5):2337-2343.

IF: 2.09

Further publications

Molnar R, Szabo L, Tomesz A, Deutsch A, Darago R, Raposa BL, Ghodratollah N, Varjas T, Nemeth B, Orsos Z, Pozsgai E, Szentpeteri JL, Budan F, Kiss I. The Chemopreventive Effects of Polyphenols and Coffee, Based upon a DMBA Mouse Model with microRNA and mTOR Gene Expression Biomarkers. Cells. 2022 Apr 12;11(8):1300.

IF: 7.666

Szabo L, Molnar R, Tomesz A, Deutsch A, Darago R, Varjas T, Ritter Z, Szentpeteri JL, Andreidesz K, Mathe D, Hegedüs I, Sik A, Budan F, Kiss I. Olive Oil Improves While Trans Fatty Acids Further Aggravate the Hypomethylation of LINE-1 Retrotransposon DNA in an Environmental Carcinogen Model. Nutrients. 2022 Feb 21;14(4):908.

IF: 6.706

Szabo L, Molnar R, Tomesz A, Deutsch A, Darago R, Nowrasteh G, Varjas T, Nemeth B, Budan F, Kiss I. The effects of flavonoids, green tea polyphenols and coffee on DMBA induced LINE-1 DNA hypomethylation. PLoS One. 2021 Apr 20;16(4):e0250157.

IF: 3.752

Molnar R, Szabo L, Tomesz A, Deutsch A, Darago R, Ghodratollah N, Varjas T, Nemeth B, Budan F, Kiss I. In vivo effects of olive oil and trans-fatty acids on miR-134, miR-132, miR-124-1, miR-9-3 and mTORC1 gene expression in a DMBA-treated mouse model. PLoS One. 2021 Feb 4;16(2):e0246022.

IF: 3.752

Conferences

Andreidesz K, Szabo L, Molnar R, Tomesz A, Darago R, Deutsch A, Varjas T, Ritter Zs, Szentpeteri LJ, Mathe D et al. A transz-zsírsavak súlyosbítják a LINE-1 retrotanszpozon DNS hipometilációt DMBA környezeti karcinogén modellben. 51st MEMBRANE TRANSPORT CONFERENCE POSTERS ABSTRACTS 2022.

Budan F, Szabo L, Molnar R, Tomesz A, Varjas T, Ritter Zs, Szentpeteri LJ, Mathe D, Hegedus I, Sik A et al. Az extraszűz olíva olaj csökkenti a karcinogén DMBA l1-RTP DNS hipometiláló hatását. 51st MEMBRANE TRANSPORT CONFERENCE POSTERS ABSTRACTS 2022.