microRNA 2012
International Symposium

Program & Abstracts

São Paulo, Brazil
25 - 27 March, 2012

microRNA 2012
International Symposium

25—27 March, 2012

Golden Tulip Park Plaza Hotel

São Paulo – SP - Brazil

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Program

Sunday, 25 March 2012

12.00 - 2.00 pm **Arrival**

2.00 - 6.00 pm **Workshops Session – New Technologies to the Study of MicroRNAs**
2.00 am – 5.00 pm

2.00 pm *miRNA: get the full picture using Microarrays*
Steven Su - Expression Marketing Manager, IMG, Affymetrix Inc

3.00 pm *Next-Gen sequencing at the touch of a button*
Carolina Almeida - Field Applications Scientist - Illumina

4.00 pm *The miRNA revolution: microRNA biogenesis, function and analysis.*
Julien Calais - QIAGEN Aplplication Scientist

5.00 pm *Early detection of colorectal cancer using microRNA biomarkers in patient blood sample*
Torben Helledie – Exiqon

6.30 pm **Opening of Conference**
Chair: Silvia Rogatto, Patricia Reis (Sao Paulo State University, Brazil)

Thomas D. Schmittgen (Ohio State University, USA)
Regulation of microRNA processing in development, differentiation and cancer.

7.10 pm **Keynote Lecture**
Wan Lam (BC Cancer Agency and University of British Columbia, Canada)
Human cancer non-coding RNA transcriptomes

8.00 pm Buffet

Monday, 26 March 2012

Morning Session - MicroRNAs in Cancer
8.10 am – 12.00 pm
Chair: Silvia Rogatto, Patricia Reis (Sao Paulo State University, Brazil)

8.10 am Junia V. Melo (Adelaide University, Australia)
microRNA alterations in leukemia

8.50 am Raphael B Parmigiani (Ludwig Institute for Cancer Research, SP, Brazil)
Role of microRNAs in colorectal tumor

9.20 am Silvia Rogatto (Sao Paulo State University and AC Camargo Hospital, Brazil)
Integrative analysis in cancer reveals microRNAs as potential regulators in penile carcinomas

10.00 am *Coffee break*

Monday, 26 March 2012

10.20 am Edna Teruko Kimura (University of Sao Paulo, Brazil)
microRNAs in thyroid oncogenesis

10.50 am Katia Ramos Moreira Leite (University of Sao Paulo - USP, Brazil)
Deregulated genetic pathways in urologic diseases: role of miRNAs in tumorigenesis

11.20 am Mark E. Hatley (St. Jude Children's Research Hospital, USA)
MicroRNAs in lung tumorigenesis

12.00 - 1.30 pm *Lunch and Technical Presentation – Life Technologies*

1) Life Technologies microRNA portfolio: From discovery to validation
Guilherme Mendes, PhD (Field Applications Scientist)

2) miRNA Detection and Profiling using Anti-miRNA Beads
Tom Xu (Principal Scientist)

Evening Session – MicroRNAs in Plants
1.30 pm – 4.15 pm
Chair: Chair: Fabio Nogueira (Sao Paulo State University, Brazil)

1.30 pm Fabio Nogueira (Sao Paulo State University, Brazil)
Small RNA-regulated pathways associated with vegetative and reproductive organ development

2.10 pm Marja Timmermans (Cold Spring Harbor Laboratory, New York, USA)
Epigenetic mechanisms in cellular differentiation and the role of small RNAs in plant development

2.50 pm Paulo Ferreira (Federal University of Rio de Janeiro - UFRJ, Brazil)
Epigenetic regulation of the sugarcane genome

3.20 pm Javier Palatnik (Universidad Nacional de Rosario, Argentina)
Biogenesis and action of plant microRNAs

3.50 pm *Coffee break/Poster*

Evening Session – Bioinformatics and MicroRNAs
4.10 pm – 5.30 pm
Chair: Fabio Nogueira, Patricia Reis (Sao Paulo State University, Brazil)

4.10 pm Renato Vicentini (Centro de Biologia Molecular e Engenharia Genética, UNICAMP, Brazil)

Bioinformatic identification of small RNAs and targets in plants

4.40 pm Igor Jurisica (Ontario Cancer Institute, University of Toronto, Canada)
Advanced bioinformatics for microRNA target prediction and data analysis

Monday, 26 March 2012

Oral Presentation Session (Selected Abstracts)
5.30 pm- 6.20 pm

5.30 pm *Whole Genome Transcriptome Analysis Reveals miR-29a targets Involved in Self-Renewal, Apoptosis and Epigenetic Regulation, Including Central Components of Active Demethylation and Maintainance of DNA Methylation Status*
Lucila Habib Bourguignon Oliveira (University of Sao Paulo, Brazil)

5.40 pm *Identification of a Novel Prognostic microRNA Signature in Mantle Cell Lymphoma*
Patricia Reis (Sao Paulo State University, Brazil)

5.50 pm *Developmental genes network of haploid Apis mellifera male embryo*
Camilla Valente Pires (University of Sao Paulo, Brazil)

6.00 pm *miR-29a Target Components of Cell Methylation Status and Pluripotency-Related Pathways: A Step Forward in Cell Reprogramming.*
Mariane Serra Fráguas (University of Sao Paulo, Brazil)

6.10 pm *Open for Questions*

6.20 – 8.00 pm *Dinner*

Poster Session
8.00 pm- 9.00 pm

Tuesday, 27 March 2012

Morning Session – MicroRNAs in Aging, Development and Evolution
8.10 am – 12.00 pm
Chair: Robson Carvalho (Sao Paulo State University, Brazil)

8.10 am Francisco Enguita (University of Lisbon, Portugal)
MicroRNAs and aging

8.50 am Andrea Münsterberg (University of East Anglia, UK)
MicroRNA regulation and developmental timing of myogenesis

9.30 am Eric C. Lai (Sloan Kettering Institute, USA)
Evolution and functional diversity of miRNA genes in animals

10.10 am *Coffee break/Poster*

10.30 am John J McCarthy (University of Kentucky Medical Center, USA)
microRNAs in skeletal muscle plasticity

11.30 am **Closing Session**
Silvia Rogatto, Robson Carvalho (Sao Paulo State University, Brazil)

12.00 pm Departure

Abstracts

Micro-RNAs involved in epithelial-to-mesenchymal transition are altered and differentially expressed in adult renal cortex and isolated glomeruli of glomerulusclerosis programmed by low protein diet in uteri.

Sene LB[1], Moraes LN[2], Garcia GJF[2], Gontijo JAR[3], Carvalho RF[2], Boer PA[1].

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The concept of the developmental origin of adult diseases, also called "fetal programming" has consequences related to kidney dysfunction and chronic hypertension. We have demonstrated previously, in a model of gestational protein restriction, reduction of nearly 27% of kidney nephron, proteinuria, and not altered glomerular filtration rate in adulthood in parallel with podocyte hypertrophy that may lead to accelerated cellular senescence, fibroses and additional nephron loss. Epithelial-to–mesenchymal transition (EMT) has been related to many forms of chronic kidney diseases and miR-200 family and miR-192 expression could contribute to the MET. Additionally, studies have showed that miRNA profiling might have relevance to the early detection of acute kidney injury and predict progression in chronic kidney disease. In this way, the objective of present study was investigate the expression of fibrosis markers and of miRNAs related to EMT in renal cortex and isolated glomeruli of adult rats submitted to gestational protein restriction. Virgin female Wistar rats were fed during pregnancy with normal-protein diet (NP 17% casein) or protein-restricted diet (LP 6% casein). Kidneys of male offspring with 16th week of age were submitted to immunohistochemistry to detect transforming growth factor (TGF)-β1, fibronectin and collagen type I expression and localization. Renal cortex and isolated glomeruli were used to study the expression profiles of miR-200a, miR-200b, miR-200c, miR-141, miR-429, and miR-192 by RT-qPCR using TaqMan MicroRNA Assays (Life Technologies, USA). 16 weeks-old LP rats presented significant increased immunoreactivity of TGF-β1, fibronectin, and collagen type I in glomeruli. These proteins were increased only in few regions of cortical peritubular matrix. In renal cortex of LP the level of miR-200c, miR-141, miR-429 and miR-192 were significantly higher. In isolated glomeruli of LP the level of miR-200a, miR-141 and miR-429 are reduced. The fetal programmed rats presented adult glomerulusclerosis that led us to suggest that miR200 family and miR-192 are up-regulated in glomeruli by TGF-β1 at early time points to induce type I collagen expression. This increase in collagen may subsequently induce glomeruli EMT by decreasing these miRNAs at later time points. We suppose that miRNAs expression may be cell-type specific and context dependent with tubular response occurring later.

Financial Support: CAPES and FAPESP.

Analysis of microRNA expression using tissues frozen and formalin-fixed paraffin-embedded from breast cancer samples.

Marino ALF[1], Macedo T[1], Longatto AF[1], Queiroz Junior AF[2], Kerr LM[2], Vieira RAC[3], Evangelista AF[4], Silveira HCS[1,5], Marques MMC[1].

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MicroRNAs (miRNAs) are small, noncoding RNAs that suppress gene expression at the posttranscriptional level via an antisense RNA-RNA interaction. miRNAs used for array-based profiling are generally purified from either frozen or fresh samples. The broadly available formalin-fixed, paraffin-embedded (FFPE) samples with their detailed clinical annotation have not been systematically investigated with respect to the quality of miRNA derived from them. It is postulated that miRNAs may be less affected than mRNAs by formalin fixation and paraffin embedding, perhaps due to their slower degradation, smaller size, and lack of poly A tails. Considering that tissues found in most pathology departments are available only in formalin-fixed and paraffin-embedded (FFPE) states, our aim was to evaluate the miRNA expression profiles of matched frozen and FFPE clinical samples using the Agilent microarray platform. The Trizol Reagent (Invitrogen) was used to extract total RNA (including miRNA) from fresh tumor samples and the RecoverAll total RNA Isolation kit (Ambion) was used to extract total RNA (including miRNA) from FFPE samples. After the total RNA was quantified with NanoDrop 2000 spectrophotometer and the quality of miRNAs was assessed using Bioanalyzer Small RNA chip (Agilent). Each miRNA sample was hybridized to arrays from Agilent miRNA array platform containing probes interrogating 866 human miRNAs. Eight cases from fresh and FFPE tissues were compared in either duplicate. Our results showed that the quality check of the frozen samples retained a miRNA peak (18- to 24-nucleotides), whereas the same peak in FFPE sample was obscured by the degraded mRNAs, transfer RNA (tRNA), and ribosomal RNA (rRNA) consistent with previous observations. Moreover the microarrays results showed consistent high correlations between matched frozen and FFPE samples (correlation R2= 0,863) supporting the use of FFPE-derived miRNAs for array-based, gene expression profiling. These results provides evidence that FFPE derived miRNAs from breast cancer tumor can be used for profiling using a microarray platform and that miRNAs appear to match information retained in frozen sample quite well.
Financial Support: FAPESP.

Downregulation of microRNA let-7b in mice insulin resistant skeletal muscle: implication of Akt isoforms balance.

Sousa TA[1], Queiroz AL[1], Silveira LR[2].

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Akt/PKB has been strongly implicated in the insulin-promoted storage of nutrients in muscle tissue. Impairment in insulin ability to maintain normal glucose homeostasis, a condition termed insulin resistance, predisposes to the development of type 2 diabetes, hypertension, and cardiovascular disease. Recently, micro-RNAs (miRNAs) have been described as non-coding molecules associated with post-transcriptional gene regulation and, therefore, regulate several physiological and pathological processes like growth, differentiation and metabolism. Our previous study shown that in diabetic rats, the miRNA let-7b regulates the protein Akt1. Thus, we suggest that an overexpression of this microRNA may contribute to insulin resistance in skeletal muscle by Akt1 regulation. Mice mioblast cells (C2C12) were treated with palmitic acid (700µM) and the insulin resistance was confirmed by evaluation of Akt phosporylation after insulin incubation by western blot. Unexpectedly, real time RT-PCR reveals a down regulation (~70%) of let-7b expression. Western blot analysis show that in mice insulin resistant cells, despite of the reduction of Akt activation (phosporylation), total Akt expression has no alteration and Akt1 is over-expressed. It has been reported differences in the roles of Akt isoforms in several processes. They are uniquely adapted to preferentially transmit distinct biological signals: whereas Akt2 and Akt3 are likely involved in insulin-stimulated glucose transport in human skeletal muscle regulating cellular metabolism, Akt1 may not be necessary for this process and seems to be involved predominantly in control of growth/proliferation instead. The mechanism underlying the apparent isoform specificity remains unclear and our results indicated that let-7b may contribute to this fine regulation.

Financial support: FAPESP.

Expression of microRNAs mir-1, mir-133 and mir-206 in skeletal muscle of rats with heart failure.

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Heart failure (HF) is a major public health problem affecting millions of patients worldwide. This syndrome is characterized by shortness of breath and fatigue at rest or with exertion and this may in part be due to skeletal muscle myopathy, with atrophy and shift from type I “slow” to type II “fast” fibers.Muscle-specific microRNAs (miR-1, -133, -206, -208, -208b, and -499) have been identified and shown to be involved in a range of processes including myogenesis (proliferation, differentiation and fiber type specification), regeneration, hypertrophy, and muscular dystrophy. These studies raised the hypothesis that the muscle-specific microRNAs miR-1, miR-133a and miR-206 would be altered during the slow-to-fast phenotype transition and atrophy in skeletal muscle of rats with heart failure.HF was experimentally induced in five adult male Wistar (HF group) by a single intra-peritoneal (ip, 60mg/kg) injection of monocrotaline (MCT, Sigma-Aldrich, Germany).Five controls rats (CT group) were injected with saline solution and were given the same quantity of food as consumed on the previous day by the treated rats. HF and CT rats were studied 33 days after monocrotaline administration when the HF group had developed overt HF. Using the histochemical reaction of myofibrillar ATPase (m-ATPase), muscle fibers types were classified as types I, IIa and Ic/IIc. There were decreased type I and increased type IIa fiber frequencies in the HF group when compared with the CT group ($p<0.05$). There were no significant changes in fiber type Ic/IIc frequencies between HF and CT groups. The cross-section area of muscle fiber types I, IIa and Ic/IIc decreased in the HF group when compared to the CT group ($p<0.05$). The expression profiles of the muscle-specific microRNAs miR-1, miR-133a and miR-206 in soleus muscle from CT and HF groups were measured by RT-qPCR using TaqMan MicroRNA Assays (Life Technologies, USA).The expression ofmiR-1were significantly decreased by -220%, however, the microRNA miR-133aand miR-206 showedno differencesbetween the experimental groups. Previous studies have shown that microRNA miR-1 collaborates to delineate the phenotype of differentiated muscle cells down-regulating a large numbers of target mRNAs, providing a mechanism by which the reduction in miR-1 may contribute to reduced MyHC, fiber shift, and atrophy in skeletal muscle during HF.

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, processes n°2011/01373-0 and n° 2010/06281-3).

Differential expression of miRNAs in Nile tilapia: investigating the role of miRNAs in sexual determination.

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MicroRNAs (miRNAs) are small non-coding RNAs of roughly 22 nucleotides, which post-transcriptionally control several biological processes. Recent tissue-specific miRNA expression studies detected sexually dimorphic miRNA expression in gonads of avian and mammals, pointing out these molecules as triggers on the sexual differentiation. Despite of advances, little is known about the expression of miRNAs related to sexual determination in fish. The Nile Tilapia (Oreochromis niloticus) is one of the most prominent farmed fish in aquaculture worldwide. Males of the species present better growing rates and high performance on fattening which make them preferably to production, and thus a priority target for uncovering sex determination mechanisms. Additionally, O. niloticus is a particularly good model for functional genomics studies because individuals are easily accessible for manipulation and the species possess its whole genome sequenced. Herein, using the Nile tilapia as a model, quantitative PCR (qPCR) was used to profile the expression of miRNAs 29b and 181a in brain and gonadal tissues of eight adult specimens (4 males and 4 females). Additionally, the snoU6 RNA was used as reference gene for normalization of expression data. The t-test was used on statistical analysis in order to find significant differences on expression levels. Both miR-29b and miR-181a are expressed in brain and gonad of O. niloticus but did not show differential expression between sexes in adults. Such lack of sexually dimorphic variation in expression indicates that these miRNAs may have a function at the adulthood probably unlinked to sex. Since the sexual differentiation in Nile tilapia take place at 5 to 6 days after fertilization, it can be hypothesized that these miRNAs keep a constant expression in both sexes after this period. On the other hand, these miRNAs presented differential expression levels in the tissues analyzed, where miR-29b ($p<0.002$) and miR-181a ($p<0.018$) were five times more expressed on brain of both sexes. Face to the preliminary results, it is necessary to expand the analysis encompassing other developmental times and miRNAs of Nile tilapia, in order to better examine the potential role of miRNAs in sexual differentiation.

Financial Support: CAPES, CNPq e FAPESP.

MicroRNA profiling of oral squamous cell carcinoma based on high-throughput sequencing.

Santana L[1,2], Paschoal AR[2,3], Torres N[1], Head and Neck Genome Project/GENCAPO[4], Durham AM[2], Severino P[1].

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Squamous cell carcinoma of the head and neck is among the leading cancers in the world, whereas a suitable prognostic marker or tailored therapy is currently unavailable. MicroRNAs could be used, in the future, to improve outcomes of this disease. MicroRNAs are small, non-coding RNAs that regulate gene expression through targeting of mRNAs. They are part of small RNA populations, which are suitable to analysis using high throughput sequencing technologies since several samples can be completely sequenced in a single instrument run for both discovery of new molecular species as well as comparison of gene expression levels. Small RNA libraries for SOLiD sequencing were prepared from 7 pairs of human oral squamous cell carcinomas and tumor-free surgical margins. We identified and quantitated microRNAs annotated in miRBase v17 dataset and, within sequences that did not map to miRBase, we searched for possible new miRNA molecules using RNA structural search (Rfam, RNAfold), prediction of ab initio (specific for the identification of miRNA) and false positive candidate detection using BLAST search against Swissprot. This approach for microRNA prediction resulted in the identification of 192 microRNA-like structures which are currently being validated. A total of 227 mature miRNAs were common to all tumor samples, and 271 to all tumor-free tissue. A preliminary analysis of differential microRNA expression between common mature miRNAsin tumor and tumor-free tissue resulted in 104 significant results. Fourteen miRNAs were up-regulated in tumor samples, some of which previously reported as deregulated in HNSCC (miR-21, miR-138, miR-31, miR-21, miR-146a/b, miR-96). Ninety miRNAs were up-regulated in tumor-free tissue.This result is in agreement with previous findings reporting overall lower levels of miRNAs in cancer as compared to normal tissues. Besides corroborating recent findings on miRNA expression profiles in HNSCC, this methodology allowed the identification of several other molecules, as well as mRNA targets possibly involved in the tumorigenic process. Deregulation of miRNAs has emerged as an important hallmark of cancer and high-throughput sequencing could become the technology of choice for the identification and quantification of these molecules.

Financial Support: FAPESP (grants 2009/04166-5 and 2005/51467-0) and Hospital Israelita Albert Einstein.

MicroRNA cluster miR-23b/27b is associated with worse outcome in penile carcinomas.

Muñoz JJ, Drigo SA, Barros MC, Busso AF, Marchi F, Guimarães GF, Soares FA, Rogatto SR .

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Penile cancer (PeCa) is a rare disease frequently treated by surgery (amputation total or partial of the organ) with subsequent psychosexual morbidity. The purpose of this study was to evaluate the expression pattern of SLCs genes (SLC1A3, SLC8A1, SLC25A4, SLC25A16, SLC25A25 and SLC27A4) and its putative regulatory miRNAs (miR-22, miR223, miR-23b, miR-26b, miR-27b and, miR-133b) in 21 PeCa and four normal penile tissues. SLC genes and miRNAs expression levels were evaluated by RT-qPCR, ACTB, GUSB and HMBS genes were used as reference genes for the SLC genes expression analysis and the RNU48, RNU47 and MIR16 were used for the miRNA expression analysis. Significant downexpression of miR-23b, miR-26b, miR-27b and miR-133b and overexpression of miR-223 were detected in tumors compared with normal controls. Significant negative correlation was observed between SLC8A1 downexpression and its predicted miRNA (miR-223, P=0.015, r=-0.482). Similarly, it was detected negative correlation between SLC27A4 overexpression ant the miR-133b (P=0.023, r =-0.454). These findings suggested that these genes are regulated by miRNAs. Interestingly, lower levels of miR-23b cluster that includes miR-27b were significantly associated with perineural invasion in PeCa (P=0,039 and P=0,032, respectively). These findings suggested this miRNA and their targets could be involved in PeCa progression, since perineural invasion is associated with an adverse outcome. To the best of our knowledge, this is the first study describing the involvement of miRNA in PeCa. Two putative target genes, SLC8A1 and SLC27A4, are likely to be regulated by miR-223 and miR-133b, respectively. Additionally, miR-23b and miR-27b are candidates for prognostic markers and therapeutic targets in PeCa.

Financial support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and National Counsel of Technological and Scientific Development (CNPq).

Searching for microRNAs: how accurate are the predictors in the model organisms.

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Identifying miRNAs plays an increasingly important role in better understanding the regulatory activities in the cell. Bioinformatics approaches can help in this task. miRNA genes prediction represents a crucial step in miRNAs functional characterization. In addition, the challenging issue remains predictions accuracy and obtain lower false positive results. Although there are a myriad prediction tools for miRNA genes, most of them is not available to use in desktop application. Even for those web servers we found limitation like the number of candidates to submit to the predictor. On the other side, there is a lack of report to help to analyze the perform of these tools in different organisms and also in a diversity of dataset . To fill this gap, we present a report that evaluated the potential contribution of the main methods available of predicting miRNA. We have tested three main tools (mir-abela, miRFinder and HHMMIR). The three predictors were tested in 9 different model organisms datasets download from latest version of miRBase database (release 18 - November 2011 - Arabidopsis thaliana (291 miRNAs), Caenorhabditis elegans (223 miRNAs), Ciona intestinalis (350 miRNAs), Gallus gallus (499 miRNAs), Danio rerio (344 miRNAs), Drosophila melanogaster (240 miRNAs), Homo sapiens (1527 miRNAs), Macaca mulatta (479 miRNAs) and Mus musculus (741 miRNAs)). To test the accuracy of these approaches we also include a diversity of non-miRNA dataset (proteins, genes/random sequences with uniform and non-uniform distribution – Total of 4011 sequences). A total of 8,705 sequences were tested. Looking for the sensibility /specificity we obtain this follow results: mir-abela (55% / 99%), miRFinder (78% / 73%) and HHMMIR (<1% /94%). Although it is possible to note the high results in specificity in two predictors, in the sensibility we cannot see the same. Also, our results showed a tendency of these predictors seems to have more accuracy to predicted mammals miRNA; and poor or average results for A. thaliana/G. gallus and C. elegans respectively. Finally, our analysis also showed that these approaches seem to have difficult to identify the mirtron class. Although there are in the literature a lot of well-known article and functionally annotated public miRNA, our results present that we still need to make improvements for miRNA predictions.

Identification of target transcripts and molecular processes modulated by miR-106a using whole-genome transcriptome analysis.

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The miR-17 microRNA family is considered tumorigenic and exerts its pleiotropic functions such as control of cell proliferation, differentiation, and apoptosis in a physiological and developmental-dependent manner. miR-106a, a miR-17 family member, is highly expressed in several types of malignances and is induced upon reprogramming of somatic cells into pluripotent stem cells (iPSC). Indeed, miR-106a-363 cluster was also found to enhance iPSC generation, indicating that miR-106a modulates key processes (such as self renewal and cell cycle control) commonly shared by cancer and normal stem cells. Despite its importance, the transcripts and pathways modulated by miR-106a remain to be uncovered. Therefore, the aim of this work was to identify transcripts targeted by miR-106a in both terminally differentiated and pluripotent cell lines. For this, we independently transfected synthetic pre-miR, inhibitory anti-miR and corresponding unspecific control molecules into human BJ fibroblasts and into pluripotent NTera2 cells. Whole-genome transcriptomes were obtained by oligonucleotide microarrays after 72 hours. In order to identify highly confident targets, transcripts downregulated by pre-miR and upregulated by the corresponding anti-miR, in both cell lines, were compared to the set of predicted targets showing evolutionary conserved miR binding sites (microrna.org). Pathways and biological processes modulated by the miRs were identified using a Functional Annotation Tool (DAVID). Among pathways with a statistically significant enriched number of miR-106a target transcripts, we identified: Regulation of Actin Cytoskeleton, Adherens Junction, Focal Adhesion, Axon Guidance and MAPK Signaling. Central components on TGF-beta pathway and MAPK signaling were also downregulated in both cell lines. In contrast, several transcripts related to pluripotency and self renewal (including Nanog and Beta-Catenin) were upregulated by miR-106a and confirmed by qRT-PCR. Our results demonstrate that several components of cancer-related pathways previously implicated in opposing pluripotency, self renewal and somatic cell reprogramming, are targeted to degradation by miR-106a. These findings contribute to our understanding on how miR-106a controls many cellular processes and may help the development of miR-based cancer therapies and possibly permit the improvement of classic iPSC generation methods.

Financial Support: FAPESP, CNPq and FINEP.

MicroRNAs array regulation in dendritic cells of mice's spleen with mRNA or DNA stimulus.

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Dendritic Cells (DC) are specialized in present and activate or inhibit lymphocytes T (LT) for way specific. These DC induced activation may be for different mechanisms, being for PAMPs or DAMPs as toll-like receptor (TLR) stimulus and inducing protein presentation. Messenger RNA (mRNA) molecules are able activate by DC, gene therapy is a very important tool for treatment in several diseases, like cancer search this stimulus is very used. But the stimulus these cells need of cell regulation avoiding a nonspecific response. The microRNAs (miRNA) are important to induce or repressing signaling pathways acting by transcription factors and others molecules. The miRNA mechanism is involved in a fine cell responses control, including cell development, differentiation and cell activation and homeostasis. These miRNAs have been identified as important regulators of gene expression like suppress the expression of specific target genes at posttranscriptional level. But the mechanisms of regulation in these cells after stimulus are not very elucidating yet. In this way, we decided knowing the difference miRNAs induced by mRNA or DNA stimuli. We used DC from spleen of Balb/c mice stimulated with 10µg of mRNAHSP65 or 10µg plasmideal DNA. The miRNA were obtained after the same stimulus and a pool of each stimulus were performed. They are incubated for 30 minutes. The pools were used in triplicate in microarray analysis using the Agilent slides and the results get using the GeneSpring and TargetScan programs. MicroRNA confirmation analysis was performed for real-time RT-PCR Taqman assay. After bioinformatics analysis we observed that stimulus with both molecules showed two differential express microRNAs, one up regulated (miR-142-3p) DNA-stimuli and miR-690 down-regulated ($p<0,05$) with mRNA-stimuli. In addition, results showed that MyD88KO mice-DC have been different expression profile of miRNA for DNA stimulus. Those molecules stimuli induce TLR interaction pattern and changing miRNA expression, being important to improve that in gene therapy had a complex regulation for induce efficient and specific immune response.

Financial support: CNPq, CAPES, FAEPA and FAPESP.

Quantification of mature microRNAs in Th2, Treg and IgG expressing memory B cells in the autoimmune disease pemphigus foliaceus.

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MicroRNAs are a class of small non-coding RNAs of approximately 22 nucleotides that act at the posttranscriptional level as important gene expression fine-tuners, through binding to the 3' UTR of mRNAs. MicroRNAs have been increasingly implicated in normal immune function and their misregulation is frequently associated to autoimmune diseases. Pemphigus foliaceus (PF) is an autoimmune disease characterized by a Th2-dependet production of IgG autoantibodies directed against skin adhesion proteins. Considering the importance of autoreactive Th2 and B cells, as well as the evidence for the involvement of regulatory T cells in PF, we aimed to verify and compare the expression patterns of five candidate microRNAs (miR-145, miR-148a, miR-155, miR-338-5p, and miR-1321) in three lymphocytes subpopulations (CD4+CD294+ Th2 cells, CD4+C25+ Treg cells and IgG expressing memory B cells [IgG+ B cells]) from five healthy controls, four patients with inactive disease (iPF) and six patients with active lesions (aPF). Our main results are: (1) in IgG+ B cells and Th2 cells, miR-155 is, respectively, 2- and 3-fold upregulated in patients with inactive disease compared to healthy controls ($P = 0.02$ and $P = 0.03$, respectively); (2) when comparing miR-155 expression in patients with inactive disease to patients with active lesions, we observed a tendency for upregulation of miR-155 in IgG+ B cells and Th2 cells ($P = 0.09$ and $P = 0.08$, respectively), suggesting a role for this microRNA in PF remission; (3) miR-148a was not detected in IgG+ B cells of patients with inactive disease, but was consistently expressed by these cells of patients with active disease; and (4) miR-1321 tends to be upregulated in Th2 cells of PF patients (iPF + aPF) compared to healthy controls ($P = 0.10$). Moreover, we found that miR-1321 levels in Th2 cells negatively correlated to those of its predicted target BLYS (BAFF), a B cell stimulator, in patients with inactive disease ($r = -0.96$) and in patients with active lesions ($r = -0.55$). Since BLyS is a cytokine known to be important for PF pathogenesis and considering the strong negative correlation found between miR-1321 and BLYS levels, miR-1321 arises as a potential microRNA for new therapeutic approaches in PF, as well as miR-155, whose upregulation in two lymphocyte subpopulations seems to be associated to PF short-ter m remission.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação Araucária (FA).

The role of microRNAs in the post-transcriptional control of T cells during the emergence of type 1 diabetes mellitus in NOD mice.

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Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by pancreatic infiltration of autoreactive T lymphocytes, which destruct insulin-producing beta cells. In the non-obese diabetic (NOD) micethis process is early as one month of age producing T1D within eight months. We hypothesized that during the development of T1D, microRNAs(miRNAs) play a role as post-transcriptional modulator upon immune reactivity associated mRNAs as thymocytes (precursor of T cells in their intra-thymic phase) mature to peripheral T lymphocytes. To test this hypothesis, we surveyed the transcriptome (mRNAs) and the miRnome (miRNAs) of thymocytes and peripheral CD3+ T lymphocytes from prediabetic or diabetic NOD mice through Agilent microarray hybridizations, which cover the whole murine functional genome. Hierarchical clustering of microarray data using the Cluster and TreeView algorithm grouped mice according to age/T1D onset and mRNAs and miRNAs according to their transcription profiling. The results showed that transcriptome and miRnome changed during the development of T cells and during onset of T1D in these animals.Moreover, we observed that several miRNAs were induced and that several immune system-related mRNAs were repressed.To evaluate the role of miRNAs and to predict mRNA targets, we used the GenMir++ algorithm to reconstruct interaction networks from the actual microarray data, for the first time in this model-system. As an example, we have found that miR-7a, miR-669h-5p and miR-712, which were inducedinteracted with several repressed immune system mRNAs including Rag1, Rorc, Sox4, Ada and Fcer2a among others.In fact we found about 25 miRNAs negatively regulating hundreds of mRNA targets, which were associated to biological processes of T cells such as immune activation, antigen processing and presentation, apoptosis, regulation of gene expression, cell communication and cell differentiation, confirming our initial hypothesis. Theseprocesses once repressed might promote long term survival of autoreactive T cells, increasing the probability of auto-immune attack of the pancreas.Interestingly, we observed that a given miRNA can interact with several mRNA targets and that a given mRNA can interact with several miRNAs at once. These results contribute to show that miRNAs play a key role in the control of biological processes in T cells in the context of auto-immunity specifically T1D in NOD mice.

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Computerized morphometric analysis of formalin-fixed paraffin embedded tissue as a tool to assess differential gene expression of microRNAs widely expressed in thyroid clinical samples.

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Papillary thyroid carcinoma (PTC) is the most common malignancy in thyroid tissue, accounting for near 80% of all thyroid cancers. Despite the generally favorable prognosis and high survival rates of most patients with PTC, some tumors display an aggressive behavior and may progress to the highly aggressive and lethal anaplastic thyroid carcinoma. MicroRNAs are a recently discovered class of small non-coding RNA molecules that take part in essential regulatory networks through targeting of mRNAs. They are reportedly involved in the pathogenesis of diverse human cancers but information regarding this association in papillary thyroid cancer is sparse. In this study, we analyzed the expression of miR-146a/b, miR-221 and miR-222, previously associated with clinical and genetic features in this kind of cancer, in formalin-fixed paraffin-embedded thyroid tissue using quantitative real-time polymerase chain reaction. The heterogeneity of embedded-tissues is a major drawback in this approach and was assessed by means of computerized morphometric image analysis. Our results corroborate the potential importance of these microRNAs in papillary thyroid cancer, although gene expression levels in tumor tissue varied from patient to patient and the expression in surrounding tumor-free areas was significant. We highlight the need for careful interpretation of microRNA expression analysis in formalin-fixed paraffin-embedded tissue for cancer biomarker discovery.

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Overexpression of miR-7 inhibits p70S6K in a head and neck squamous cell carcinoma cell line and plays a role in cell proliferation.

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MicroRNAs are small noncoding RNAs that regulate gene expression by degrading or destabilizing the RNA message or by inhibiting protein translation of specific mRNA targets. Currently, broad evidence shows that alterations in microRNA expression are involved in the initiation and progression of human cancers. MiR-7 has been shown to regulate epidermal growth factor receptor (EGFR) signaling, a matter of great interest to the head and neck squamous cell carcinoma (HNSCC) clinical setting. EGFR is frequently overexpressed in HNSCC and is an important therapeutic target. MiR-7 targets not only EGFR but also multiple other genes involved in EGFR-related tumorigenesis. Nevertheless, this mechanism has not been addressed in HNSCC. Aiming to understand the role of miR-7 in EGFR signaling under a HNSCC background, we over-expressed this molecule in an oral squamous cell carcinoma cell line and evaluated the expression level of 84 genes of the EGFR signaling pathway using a PCR Array. Among these genes, 41 were deregulated after the interference, 39 of which being up-regulated in the cell over-expressing miR-7 while 2 were down-regulated. The deregulation of the PI3K/AKT/mTOR/p70S6K1 pathway, associated with cell survival and proliferation, was observed. p70S6K1, a recently validated miR-7 target, was one of the down-regulated genes. Western blot analysis showed lower levels of p70S6K1 in miR-7-over-expressing cells and an immunofluorescence assay for proliferation analysis indicated lower levels of proliferation of these cells. Our results suggest that miR-7 can interfere in the regulation of PI3K/AKT/mTOR/p70S6K1 through directly targeting p70S6K1.

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Expression patterns of miRNAs and their predicted target genes in fat body of reproductive and non-reproductive honeybee (*Apis mellifera*) queens.

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In honeybee colonies, queens are engaged in reproduction, activating their large ovaries after mating. Workers, by contrast, are normally sterile, but they maintain functional ovaries, which could be activated in queenless condition. In order to identify potential genetic interactions involved in the regulation of reproduction in honeybee females, we predicted a gene network based on in silico searching between 35 miRNAs obtained by Illumina deep-sequencing of activated ovaries from workers and the 3´untranslated region of coding-genes expressed in fat body and related to ovary activation process. Mapmodulin, tyramine receptor and miR-316 were the most connected nodes. In order to validate some of identified molecular interactions, we investigated the expression profiles of eight miRNAs and six putative target genes in fat body samples of virgin and egg-laying queens. In general, the miRNAs levels were higher in egg-laying queens compared to virgin ones, but significant differences were observed only for the miR-184 and miR-275. Concerning the target genes, we observed that mean levels of Mlc-2 and Ubq were significantly higher in fat body of non-reproductive queens, in an antagonistic manner to expression of their predicted regulatory miRNAs, miR-278 and miR-375. Together, our computational and experimental findings support the existence of molecular pathways governing ovary activation in both castes, and open new ways for investigations related to honeybee reproduction.

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Overexpression of miR-696 contributes to insulin resistance by downregulation of mitochondrial biogenesis.

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Insulin resistance is a feature associate to type II diabetes but its mechanism is not fully understood. Recently, micro-RNAs (miRNAs) have been described as non-coding molecules associated with post-transcriptional gene regulation and, therefore, regulate several physiological and pathological processes like growth, differentiation, metabolism, cancer and diabetes. It is known that the miR-696 regulates Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1α), involved in mitochondrial biogenesis. We suggested miR-696 may be involved at insulin resistance of skeletal muscle cells. Mice myoblast cells (C2C12) were treated with palmitic acid (700μM) for 24 hours. The insulin resistance was confirmed by evaluation of Akt phosporylation after insulin incubation by western blot. Expression of mature miR-696 was evaluated by real-time RT-PCR using TaqMan® MicroRNA Assay. The expression of microRNAs was normalized with the SnoR-202. Gene expression of PGC1α was evaluated by real time RT-PCR using Sybr Green. Analysis of oxygen consumption was performed by oxygraph (Hansatech Instruments, Ltda) using FCCP and oligomicin as controls. We observed that in insulin resistant cells the miR-696 expression was marked elevated (~3-folds). Insulin resistant cells also shown a decreased expression of PGC1α mRNA indicating an association of miR-696 with mitochondrial biogenesis. We also observed the reduction of oxygen consumption in C2C12 insulin resistant cells indicating reduction of mitochondrial respiration. Therefore, we suggest that miR-696 contributes to insulin resistance by downregulation of mitochondrial biogenesis and activity.

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miR-26a and miR-26b are associated with triple-negative breast carcinomas.

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MicroRNAs are small non-coding RNA molecules (18-24 nucleotides in length) that regulate gene expression at the posttranscriptional level. These endogenous molecules play critical roles in regulating normal developmental processes, but when deregulated, are causally linked to the pathogenesis of numerous diseases. The miR-26 family, which comprises miR-26a and miR-26b, has been described as deregulated in diverse tumours, including breast carcinomas. Breast cancer is a complex disease and although recent researches have emphasized the heterogeneity of the disease, crucial questions in biological mechanisms remain poorly understood. Aiming to investigate miR-26a and miR-26b in breast carcinomas and their association with clinical and histopathological parameters, miR-26a and miR-26b expression levels were evaluated by RT-qPCR in 63 ductal invasive breast cancer samples and five normal breast tissues. Significant downexpression of miR-26a and miR-26b were detected in tumour samples when compared to normal breast tissues (P=0.003 and P=0.014, respectively). ER-negative tumours showed a significant downexpression of miR-26a and miR-26b (P=0.006 and P=0.003, respectively). Similarly, triple negative tumours showed a significant downexpression for both miR-26a and miR-26b when compared to other subtypes (P=0.020 and P=0.004, respectively). Decreased levels of miR-26a and miR-26b were also observed according to increased tumour grade (P = 0.008). In addition, significant positive correlation was observed between these microRNAs (r=0.889; P<0.001). These results indicated that miR-26a and miRNA26b are involved in breast carcinogenesis. The functional significance of these findings and the regulatory mechanism involved in this process is crucial to explain the association with ER, tumor grade and, mostly, with triple negative breast carcinomas.

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Low expression of microRNAs not located on chromosome 21 in peripheral blood mononuclear cells of children with Down syndrome associated with biological processes relevant to DS pathogenesis.

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Trisomy 21 is the genetic basis of Down syndrome (DS), the most common human chromosomal disorder. It is believed that the over-expression in about 50% of a specific gene or a group of genes located on chromosome 21 present in triplicate in DS individuals is directly responsible for DS features. In addition, there are evidences that secondary effects of trissomic genes affect multiple metabolic pathways, resulting in cellular dysfunction. More recently, studies have shown that trisomy 21 results in the over-expression of microRNAs, which could result in low expression of specific proteins and contribute to DS phenotype. Thus, the objective of this study was to identify differentially expressed microRNAs in peripheral blood mononuclear cells of DS and non-DS children and to identify biological processes relevant to DS pathogenesis associated with predicted gene targets of microRNAs differentially expressed. Mature microRNAs were quantified from peripheral blood mononuclear cells of six children with free trisomy 21 and six control children using TaqMan® Low Density Arrays (Applied Biosystems), which enable the quantification of 754 mature microRNAs. The target prediction was performed using the software TargetScanHuman v. 5.2. Information about gene targets was obtained using the software Bioprocess, a database that obtains data from the National Center for Biotechnology Information (NCBI). Of the 490 mature microRNAs expressed in the samples, 49 were low-expressed in DS group. The microRNAs located in chromosome 21 did not present differential expression between the groups. Bioinformatics analysis showed that genes involved in several relevant biological process to DS, including apoptosis, reactive oxygen species metabolism, mitochondrial metabolism, immune system, cell aging, cycle and division and control of gene expression, are predicted targets of microRNAs differentially expressed in DS children. So, we conclude that DS children present low expression of microRNAs not located on chromosome 21 in peripheral blood mononuclear cells and biological processes relevant to DS pathogenesis are associated with predicted gene targets of these microRNAs differentially expressed.

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Identification of microRNA486-5p as a K-Ras target in lung cancer.

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K-Ras–induced lung cancer is a very common disease, for which there are currently no effective therapies. Direct targeting of K-Ras has failed in clinical trials and intense efforts are underway to identify K-Ras targets that play a crucial role in oncogenesis. One promising K-Ras-regulated pathway that has so far been overlooked is the microRNA pathway. Even though many microRNAs that regulate expression of K-Ras are known, microRNAs regulated by K-Ras remain largely unknown. Our goal was to identify microRNAs regulated by oncogenic K-Ras in lung cells that could contribute to the oncogenic phenotype. Due to a reported positive correlation between microRNA486-5p (miR-486-5p) expression and the presence of K-Ras mutations in colon cancer specimens, we decided to investigate in lung cells whether K-Ras regulates miR-486-5p. For that purpose we used an immortalized human primary lung epithelial cell line (SALEB) and its isogenic K-Ras-transformed counterpart (SAKRAS). We found that, when compared to SALEB cells, SAKRAS cells express miR-486-5p at a significantly higher level. SAKRAS cells also express lower levels of the miR-486-5p targets CADM1, BTAF1, PTEN and FoxO1. In order to confirm our results in cell lines derived from lung cancer patients, we used K-Ras positive lung cancer cell lines H358 and A549 to generate stable lines with doxycycline-inducible expression of two different short hairpin RNAs targeting K-Ras. We then evaluated how inhibition of K-Ras expression in these cells affected miR-486-5p expression. Inhibition of K-Ras expression led, not only to decreased expression of miR-486-5p, but also to higher protein levels of miR-486-5p target FoxO1. Taken together, these results indicate that miR-486-5p is a K-Ras target in lung cancer, which leads to attenuated expression of miR-486-5p targets, such as PTEN and FoxO1. Regulation of miR-486-5p targets by K-Ras could contribute to cell survival and transformation. Further understanding of miR-486-5p targets could uncover novel pathways for K-Ras-induced lung cancer therapy design.

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miR-29a Target Components of Cell Methylation Status and Pluripotency-Related Pathways: A Step Forward in Cell Reprogramming.

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Induced pluripotent stem cells (iPS) are retroviral-transduced cells (by introduction of Oct4, Sox2, Klf4 and c-Myc transcription factors), functionally and phenotypically similar to embryonic stem cells (ESC). However, the reprogramming efficiency is extremely low and transduced-retrovirus integrates to the host genome, invalidating the clinical use of iPS. Among alternatives for the potentially harmful genetic modifications currently used, microRNAs (miRs) have emerged as a promising field. In fact, the ectopic expression of specific miRs preferentially expressed in pluripotent stem cells (SC), were shown to improve reprogramming carried by the classical factors, or to completely substitute them. Among these, miR-29a transcription was shown to be directly repressed by cMyc during early reprogramming and its inhibition (by anti-miR) reduced p53 and ERK1/2 levels. Despite these punctual results, a broad and systematic evaluation of how signaling pathways are regulated by miR-29a, is still unknown. With that in mind, in order to explore the molecular mechanisms by wich miRs-29a contribute to iPS reprogramming, synthetic pre-miRs, inhibitory anti-miRs and corresponding unspecific control molecules were independently transfected into human BJ fibroblasts and into pluripotent NTera2 cells. After 72h, gene expression profiles were obtained by oligonucleotide microarrays. Confident targets were identified by comparing to the set of transcripts downregulated by pre-miR transfection and upregulated by the corresponding anti-miR (in both cell lines) to that of predicted targets, showing evolutionary conserved miR binding sites. Reprogramming-associated molecular changes induced by synthetic pre/anti-miR-29a were compared to the changes observed during iPS reprogramming, to identify signaling pathways potentially relevant for the reprogramming process. For this, two fibroblast/iPS transcriptome sets were used (a partially reprogrammed iPS generated by our group and that of Takahashi, obtained by OSKM factors insertion). Pathways and biological processes modulated by the miRs were identified using the DAVID Tool and selected targets were confirmed by qRT-PCR. Among pathways with a significantly enriched number of confident miR-29a targets, we found: anti-apoptotic pathway, upstream of p85a, leading to BAD phosphorylation (ERK2); Wnt signaling; chromatin remodeling and active DNA demethylation (TET1 and TDG) components. Importantly, Tet1 gene is directly controlled by Oct4 and Sox2 and is induced concomitantly with 5hmC (5-Hidroxymethylcytosine) during cell reprogramming. Moreover, promoters of pluripotency factors are enriched for 5hmC and bound by Tet1. Tet1 knockdown in mouse ESC decreases total 5hmC levels, increases DNA methylation at the Nanog promoter and reduces its expression, impairing proliferation. We show that several transcripts targeted by miR-29a are components of pathways that contribute to pluripotency, self-renewal and iPS reprogramming. Our findings contribute to the understanding of how microRNA modulation contributes for iPS reprogramming improvement. Financial Support: FAPESP / CNPq.

Role of Jhe and its regulatory molecules in honey bee (*Apis mellifera*) development and caste differentiation.

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Juvenile hormone (JH), along with 20-hydroxyecdysone (20E), regulates metamorphosis and insect development, and plays an essential role in honey bee (Apis mellifera) caste determination. In this social species, female caste dimorphism is governed by differential feeding of queen- versus worker-destined larvae during development. Nutritional stimulus triggers an endocrine response characterized by high titers of JH in prospective queen larvae, while titers remain comparatively low in worker destined larvae. Though worker- and queen-destined larvae share a common genotype, differential hormone activity during development results in markedly different gene expression between them. In both castes, at precise developmental time points, JH is released or removed from the hemolymph in a process that can involve its synthesis or degradation. JH esterase (JHE) is one of the main enzymes responsible for JH degradation and possibly plays a crucial role in caste determination. To clarify JHE's involvement in this process, the gene that encodes JHE was knocked down using an RNAi protocol. A group of larvae was fed dsRNA (double-stranded RNA) for the jhe gene and showed a 40% decrease in the mRNA levels of this gene. In addition, larval development was a little delayed, indicating that other processes were affected by jhe knockdown. The control group, that was not fed with dsRNA, developed normally. To further clarify the mechanisms and genes regulating expression of jhe during caste determination, we conducted a computational prediction (RNAhybrid, free energy livre ≤ -15 Kcal) to identify micro RNAs (miRNAs) predicted to bind to sites in the jhe 3' UTR (untranslated region). Amongst other miRNAs, mir-8 and mir-278 were both predicted to bind to the selected region. In other organisms, mir-8 is involved in controlling body size and cellular growth and mir-278 is related to energetic homeostasis and apoptosis inhibition. These processes are closely related to caste determination and differentiation in honey bees, and sensitive to the JH titers. By analyzing the expression profiles of these miRNAs, we observed a decrease in mir-8 levels in individuals treated with dsjhe and an increase in mir-278 levels in the treatment group relative to controls, suggesting that both miRNAs may be involved in regulating jhe gene expression, however in an antagonistic way. Taken together these results indicate a possible role for jhe and its regulatory molecules in the important process of caste determination in A. mellifera.
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MicroRNAs Expression Are Modulated In A Model Of Left Ventricular Hypertrophy Induced By Voluntary Exercise In Mice.

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Physiological and pathological left ventricular hypertrophy (LVH) are distinct processes that have differential patterns of gene expression. MicroRNAs are small non-coding RNAs that regulate gene expression. The aim of this study was to evaluate the expression of microRNAs in mice hearts subjected to physiological LVH. LVH was induced by voluntary exercise in running wheels. Balb/c mice were kept in cages with exercising wheels (Exercise group – EXE; n=14 sacrificed on day 7 and n=16 sacrificed 35 days after training) or in cages without wheels (Sedentary group – SED; n=12 in each time point). LVH was evaluated by left ventricular weight/body weight ratio (LVW/BW). LV mass was also estimated by echocardiography using an EnVisor HD System (Philips Medical, Andover, MA, USA). A microRNA microarray based on the MIRBASE version 16 (LC Sciences, Houston, Texas, EUA) was carried out using a pool of RNAs (n = 4 per group) extracted from the LV. Comparisons between groups were performed by Student t test. After 7 days of training there was an increase of 17% in LVW/BW ratio on EXE compared to SED group (3.8±0.1 vs. 3.3±0.1, respectively; p=0.0004) and this result was sustained at 35 days (18% increase; 3.9±0.2 vs. 3.3±0.04, respectively; p=0.002). Echocardiography based LV mass was increased in the EXE [35 days] group when compared to the SED [35 days] group (58±5.0 vs. 34±16 mg, respectively; p=0.01). In the microarray analysis we observed that the EXE [7 days] group had 35 deregulated microRNAs and the EXE [35 days] had 25 deregulated microRNAs when compared to the SED groups (p value<0.01 for both analyses). The most upregulated miRNAs were miR-341* and miR-1224 and the main downregulated miRNAs were miR-21, miR-26b, miR-150 e miR-499. We selected miRs that have been previously described to be involved in cardiovascular diseases (miR-21, miR-195 and miR-499) and also, deregulated miRs from microarray (miR-26b, miR-150 and miR-341) to analyze their expression by qRT-PCR. In accordance with microarray data, miR-26b expression was decreased in EXE [7 days] (p = 0.02) and miR-150 showed a decrease in EXE [35 days] (p = 0.02) compared to the respective sedentary group. There was no significant change in expression of miR-21, -195 and -499 when comparing the EXE and SED groups in both time points. Physiological LVH was associated to significant modulation of heart microRNA expression. Our data suggest that miRNAs expected to be involved in regulatory pathways of pathological hypertrophy are not deregulated in physiologic cardiac growth. These data might help to understand the specific role of microRNAs on cardiac adaptation.

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Whole genome transcriptome analysis reveals mir-29a targets involved in self-renewal, apoptosis and epigenetic regulation, including central components of active demethylation and maintainance of DNA methylation status.

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The function of microRNAs (miRs) in hematopoietic stem cells (HSC), and leukemia stem cells (LSC) is poorly understood. Recent studies revealed miR-29a as an important player in the regulation of HSC and (LSC). A preliminary database and literature search revealed that, in normal cells, the highest levels of miR-29a are found in T-cells, followed by B-cells and then by granulocytes, monocytes and CD34+ HSC; while in leukemic cells, highest levels are found in indolent Chronic Lymphocytic Leukemia (CLL), followed by aggressive CLL (at levels comparable to B-cells), Mantle Zone Lymphoma (MZL), and (at levels comparable to HSC), by B-cell and T-cell Acute Lymphoblastic leukemias (ALL) and Acute Myeloid Leukemia (AML). Overall, these studies associate to lower miR-29a levels to a more aggressive disease and a worst prognosis (CLL, MZL and AML). In line, restoration of miR-29 in primary AML blasts or cell lines induces apoptosis and dramatically decreased tumor growth in xenograft models. Intriguingly, overexpression of miR-29 in mouse B-cells or HSC results, respectively, in the development of leukemia with B-CLL characteristics or, AML, by converting myeloid progenitors into self-renewing LSC. To shed further light on the role of miR-29a in self-renewal and leukemogenesis, we carried systematic identification of transcript targets. Synthetic pre-miR-29a, inhibitory anti-miR-29a and respective control molecules were independently electroporated into Jurkat cell, using the Neon System. 48h post-transfection gene expression profile was obtained using Agilent microarrays. Transcripts simultaneously down-regulated by the pre-miR and up-regulated by the corresponding anti-miR, were compared to the set of predicted targets showing evolutionary conserved miR binding sites to identify confident targets. To identify pathways and biological processes modulated by the miR-29a, we used a Functional Annotation Tool (DAVID) and selected targets were validated using qRT-PCR. Among pathways with a statistically significant enriched number of miR-29a target transcripts, we identified: Apoptosis (FAS, BIRC2), WNT (WNT8B/16, FZD4/10, LRP6, TCF7L1), TGF-beta (TGFB3, ACVR2A/B, BMP8A, SMAD2, BMPR1A/B, LEFTY2,), Jak/Stat (LIF, LIFR, SPRY1) and cancer (MDM2, APC, NRAS, PTEN, PTENP1, RARB, FOS). Further inspection revealed central components of active demethylation (including TET1/2/3 and TDG) and maintenance of DNA methylation following cell division (DMNT3b). qRT-PCR confirmed the significant down-regulation of TET1/2/3 and DMNT3b after pre-miR transfection ($p < 0.05$, $n=3$). Both, oncogene hypomethylation or tumor suppressor hypermethylation can lead to oncogenic transformation. For instance, mutations in the DNMT3A (leading to hypomethylation) or the TET family (leading to hypermethylation) are found in a significant fraction of myeloid disorders. Interestingly, Tet2 haploinsufficiency in the hematopoietic compartment leads to increased stem cell self-renewal and myeloproliferation, paralleling the effects of miR-29a overexpression. Our results indicate that miR-29a may have opposing effect depending on the context, and sheds light in its roles in normal and leukemic stem cells.
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Differential expression of miR-499 between white and red muscle fibers in Nile tilapia fish.

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The Nile Tilapia Oreochromis niloticus is the one of the most important farmed fish in freshwater aquaculture globally. The male individuals are the most profitable to the productive sector by presenting higher growth than females. However, little is known about the molecular mechanisms that control and regulate the pathways responsible for muscle growth in O. niloticus. Recently, a class of small non-coding RNA molecules, the microRNAs (or miRNAs), have been shown to be essential in the regulation of specific cell pathways of several vertebrates, including the biology of skeletal muscle. Thus, it can be hypothesized that miRNAs also play a key role on skeletal muscle growth and differentiation in Nile tilapia. Aiming to test for this hypothesis we performed qPCR experiments to check the miRNA expression profile in distinct types of muscle fibers (red and white muscle) and between male and female specimens. Our analysis, carried out on five muscle-specific miRNAs (miR-1, -133a, -133b, -206 and -499) in adults of Nile tilapia, revealed a highly differential expression of miR-499 between white and red muscle fibers. We could not find any significant sex-biased differential expression of miRNAs in the red and white muscles analyzed. Nevertheless, the miR-499 was up regulated (RQ = 43.647; p = 0.001) in the red muscle fiber (slow-twitch) in comparison to the white muscle fiber (fast-twitch). Such expression profile suggests miR-499 can be involved in muscle specification and maintenance in O. niloticus. These results are in consonance with recent studies in mouse and zebrafish, which have described the participation of miR-499 in the process of muscle fiber specification through the regulation of Sox6 and Rod1 genes. Specifically, Sox6 have been shown to drive the terminal differentiation of fast-twitch fiber by suppressing the transcription of slow- twitch fiber specific genes, whereas Rod1 expression is up-regulated in the initial differentiation of fast-twitch muscle fiber. Thus, we speculate that miR-499 can be essential for the differentiation and maintenance of the slow-twitch muscle fiber through down-regulation of Sox6 and Rod1 genes. Further experiments on the expression of Sox6 and Rod1 genes in Nile tilapia are under development to help make clear the biological significance of miR-499 regulation.

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Evidences for biogenesis pathway of tRNA-derived fragments (tRFs) in plants.

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Specific fragments of 19 nucleotides derived from tRNAs have been reported to be generated under stress conditions in several organisms. A number of these fragments (named tRFs) have also been observed at basal levels in human and plant cells under normal growing conditions. Although some evidences suggest that tRFs are implicated in cell differentiation and translation, little is known about their biogenesis. We have identified differentially expressed tRFs in sugarcane small RNA libraries from axillary buds and young leaves. One of those, namely tRF1, was the highest expressed tRNA-derived fragment in sugarcane young leaves. We cloned this fragment and confirmed its 19-nt sequence that seems to be generated from a 5'-halve of a chloroplast-specific tRNA. We analyzed its accumulation level via pulsed stem-loop RT-PCR in leaf tissues from one-, two-, and four-week-old sugarcane plants. We were able to consistently detect tRF1 transcripts using this technique and showed that its expression is similar throughout those stages of leaf development. Next, as tRF1 sequence is highly conserved among several plant species, we analyzed the accumulation of Arabidopsis tRF1 in leaf tissues of several siRNA/microRNA biogenesis-associated mutants. We found that in three of the mutants, dcl1, dcl3, and rdr2, the 19-nt tRF1 transcripts almost disappear. These results indicate that some tRNA-derived fragments may be Dicer-dependent in plants, as it has been shown for tRFs from HeLa cells. More experiments are needed to confirm these evidences for tRFs biogenesis in plants.

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Synthetic siRNA-FAM detection method.

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Since RNAi was discovered it has become a major subject of interest in biological research and in therapeutic approach for gene-related diseases. Despite the therapeutic potential of siRNA, the quantification, usually, is based on the effect promoted by this therapy. The present study related a method for detecting and quantifying a synthetic siRNA labeled with FAM. FAM is a dye with fluorescence properties. The spectral properties and responses to the various pH were analyzed in spectrophotometer and microplate reader. These results were compared with the properties of fluorescein. siRNA-FAM showed the same properties of fluorescein, maximum absorption on 490 nm and maximum emission on 512 nm, resulting the same fluorescence spectral of fluorescein. In both cases, the fluorescence was more intense when the pH is higher. For siRNA-FAM, when water was the solvent (pH 6.2) the quantification limit was 0.02 µM; and when solvent was HEPES buffer (pH 7.4) this value was 6 nM. The response of fluorescein was very similar, but the fluorescence intensity was larger (around 4 times). Therefore, the analytical curve has a lower quantification limit. Through these results we can conclude that the siRNA labeled with FAM has the same properties of fluorescein in fluorescence analysis, since the FAM is a derivative of fluorescein. Thus, the use of fluorescein in preliminary tests can determine the best conditions of analysis for the siRNA-FAM. The use of fluorescence is appropriate in the direct quantification of the siRNA-FAM, instead of the cell response.

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Drug delivery system for RNA interference: liquid crystalline dispersions functionalized with hyaluronic acid.

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RNA interference (RNAi) technology allows the use of genes as drugs and may provide treatment to several illnesses, both congenital and acquired diseases. Small interference RNA (siRNA), a double stranded RNA with ability to block specific proteins synthesis, has been proposed as a therapeutic agent in many studies, but the main hurdle is to make it suitable to clinical treatment in an effective and safe delivery system. We developed a topical formulation aimed to deliver siRNA to the cornea, the foremost tissue of the eye. The delivery system is composed of liquid crystals of monolein (MO) in association with polyethylenimine (PEI), a cationic polymer used for siRNA vectorization, and the polianionic hyaluronic acid (HA) as a cellular receptor mediator aimed to target epithelial cells of the cornea. The MO was melted at 42°C and mixed with PEI for 3 minutes using a vortex mixer (Ika®), then TRIS buffer pH 6.5 containing 1.5% poloxamer 407 was added and left at rest overnight to stabilize the liquid crystalline phase. The liquid crystalline phases were observed in optic microscope Axioplan 2 (Carl Zeiss AG, Germany) with polarized filter. The formulation composed of MO/PEI/water phase (20:0.04:79.6 w/w/w) was dispersed using a ultrasonic processor MicrosonTM XL-2000 (Misonix Inc., USA) for 2 minutes. The dispersion of liquid crystals was complexed with siRNA (2.5 uM) for 30 minutes and 0.02% of HA was added and left at rest for 30 minutes. The size and surface charge of the system was evaluated using a Zetasizer Nano System ZS (Malvern Instruments, Germany), resulting in 243.7 (±1.5) nm average size and charge of +0.79 (±0.28). The low positive charge of the formulation can be attributed to HA anionic charge and the nanometric size obtained is a desirable characteristic of the delivery system. The electrophoreses of the formulation and controls showed ability to complex with siRNA, even with low positive residual, therefore it could protect siRNA form degradation and act as a gene carrier. The cellular uptake was performed in L929 fibroblasts, which are used as a model of CD44 receptor expression for hialuronic acid, with a siRNA labeled with fluorescein (FAM, excitation e emission a 492/517 nm) and the transfection efficiency was observed in a fluorescence microscope (Carl Zeiss, Germany) with emission of 505-565 nm and excitation of 480-520 nm. The efficiency of transfection of siRNA-FAM was lower in the association of MO/PEI/HA than with MO/PEI alone, probably due to the high molecular weight of hyaluronic acid. Even though HA did not improve the cellular uptake, a low molecular weight polymer might lead to satisfactory results.

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MicroRNA and mRNA expression profile of operable oropharyngeal squamos cell carcinoma according to prognosis.

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Little is known about the integration of microRNA and gene expression profiles in oropharyngeal cancer (OSCC). A cohort of 15 patients was prospectively identified with a histological diagnosis of OSCC, submitted to surgery with curative intent. Two prognostic patient groups were defined, according to overall survival (short x long), with a 24 month cut-off. These groups were matched by known prognostic factors: TNM staging and histological grade. We used the TaqMan Low Density Array (TLDA) Human MicroRNA Panel A and Affymetrix HG-U133A Plus-2 GeneChipTM arrays for global miRNA and mRNA expression analysis, respectively. microRNA and genes differentially expressed between poor prognosis tumors compared to normal tissue versus good prognosis tumors compared to normal tissues groups were identified using student T test ($p < .05$). Based on TargetScan (http://www.targetscan.org/) predicted target/miRNA pairs Pearson correlation was calculated. Nineteen target/microRNA pairs were identified including genes known to play important roles in head and neck cancer like SERPINE1 (PAI-1)/hsa-miR-449b and SERPINE1 (PAI-1)/hsa-miR-199b-5p, CDK6/hsa-miR-449b and CDK6/hsa-miR-502-5p. In addition ARPC1B/hsa-miR-124 and ARPC1B/hsa-miR-338-5p were associated for the first time with bad prognosis in OSCC. HLF, an oncogenic transcription factor, previously associated with leukemia and thyroid cancer was inversely correlated with four microRNAs: hsa-miR-454, hsa-miR-19a, hsa-miR-301a and hsa-miR-20a. These associations indicate that microRNAs may be biomarkers related to prognosis or possible targets for drug modulation in oropharyngeal cancer.

Role of microRNAs in the lungs of infected mice with *Mycobacterium tuberculosis*.

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MicroRNAs can be considered molecules that regulate gene expression in multicellular organisms. Mature microRNAs are derived from a long primary transcript, from genome, which undergoes a series of maturation stages processed by endonucleases, until they become mature, inhibiting translation of mRNA target. The role of microRNAs is little described in tuberculosis disease, thus, the aim of this work is to determine the pattern of microRNA expression in the lung of infected mice with Mycobacterium tuberculosis (Mtb). Tuberculosis (TB) is an infectious disease caused by the bacillus Mtb, remaining as the second leading cause of death from infectious diseases worldwide. The immune response in TB has shown a Th1 pattern with IFNγ production. The detection of microRNAs in acute and chronic phase of infection could help to clarify some immunological mechanisms and to characterize microRNAs as markers in disease. BALB/c mice were infected intranasally (i.n.) using 100 µl of suspension containing 1x105 bacilli suspension of Mtb H37Rv. After 30 and 60 days of infection, total RNA of the lungs of infected animals and controls were extracted using the TRIzol (Invitrogen, USA). The identification of microRNAs was accomplished through Agilent miRNA microarray system and analyzed by GeneSpring GX11.5 software. We identified 27 and 24 microRNAs differentially expressed on 30 and 60 days, respectively. 9 microRNAs were down regulated and 18 microRNAs were up regulated in the lungs of infected mice on 30 days post-infection. Similar results were found on 60 days post-infection, 12 microRNAs were down regulated and 12 microRNAs were up regulated in the lungs of infected mice. qPCR analysis were performed to confirm the expression of some microRNAs, according to their expression and relevant published datas. The expression of these microRNAs: miR-21, miR-135b, miR-208a, miR-142-3p, miR-706, miR-146a, miR-146b and miR-155, were confirmed by Real Time PCR. Analysis of these microRNAs found in the lung of infected mice and their possible gene targets are being developed using specific prediction programs.

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TNRC6A mRNA levels is related to prostate cancer occurrence.

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TNRC6A gene codifies a phosphoprotein that belongs to GW182 family acting in the RISC complex and involved in the posttranscriptional silencing of genes by miRNAs. In human cancers, the mature miRNAs may suppress or promote tumor development as well as the proteins associated with RISC complex. The quantitative fluorescence by SYBR Green I fluorophore analyses in Real Time PCR was used to quantify TNRC6A mRNA expression in cells from prostate tissue with prostate cancer (PCa, n=51) and benign prostatic hyperplasia (BPH, n=15) to investigate whether the TNRC6A gene expression in mRNA levels could be associated with occurrence and progression of cancer. Using B2M transcripts as endogenous control, the average of the TNRC6A relative quantification (RQ) was approximately three times higher in PCa group. None correlation was observed among RQ TNRC6A and clinical parameters as TNM system classification or Gleason score. Nearly 63% (32 out of 51) and 20% (3 out of 15) PCa and BPH cases presented TNRC6A mRNA expression higher or equal than 1.24 value, a cut off value established after ROC curve analyses. A significant association was found for prostate cancer and RQ TNRC6A higher than cut off value having approximately 6-fold higher chances for PCa occurrence compared to benign cases, with 58% of sensibility and 80% of specificity. The results indicated that quantification of TNRC6A expression is higher in PCa compared to BPH patients and a more extensive analysis can determine if the TNRC6A mRNA levels could be used as a biomarker for prostate cancer occurrence.

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Angiotensin II promotes skeletal muscle angiogenesis induced by exercise training: role of microRNAs-27a and -27b.

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Exercise training (ET) promotes skeletal muscle angiogenesis related to high performance; however, the underlying molecular mechanisms are unknown. MicroRNAs (miRNAs) are an emerging class of non coding small RNAs that regulate post-transcriptionally the expression of their target genes. MiRNAs-27a and -27b target the angiotensin-converting enzyme (ACE). We investigated, for first time, the effects of ET on soleus miRNAs-27a and 27b expression and whether they regulate the skeletal muscle renin angiotensin system (RAS) in ET-induced angiogenesis. Wistar rats (n=30) were assigned to 3 groups: sedentary (S), Trained 1 (T1) and Trained 2 (T2). T1: swimming training consisted of 60 min of duration, 1x/day/10 weeks, with 5% caudal body weight workload. T2 the same as T1 until 8th week, in the 9th week they trained 2x/day and in the 10th week 3x/day. Blood pressure (BP) and heart rate (HR) were evaluated by direct measurement and angiogenesis by soleus capillary-to-fiber ratio. Soleus miRNAs-27a and -27b were analyzed by real-time PCR and ACE activity and protein expression by fluorometric method and western blotting, respectively. Soleus angiotensin II (ANG II) and VEGF concentration were evaluated by ELISA. Angiotensinogen (AGT) and ANG II type 1 (AT1) receptor protein expression also were measured. RAS involvement in the skeletal muscle angiogenesis induced by ET was analyzed using AT1 receptor blockade (Losartan- 20 mg/kg/day) during ET protocol. One-way ANOVA with Tukey post hoc test were performed. ET was effective in increasing exercise tolerance, peak oxygen uptake and soleus citrate synthase activity in an ET volume-dependent manner compared with S group. BP was unchanged while resting HR decreased in all trained groups. Skeletal muscle angiogenesis obtained by T1 and T2 was 87% ($p<0.01$) and 137% ($p<0.001$), respectively. In contrast, Losartan prevented the soleus angiogenesis in both trained groups. Soleus miRNA-27a levels decreased in both trained groups (23% in T1 and T2, $p<0.05$) compared with S group. Similar, miRNA-27b reduced 23% in T1 ($p<0.01$) and 32% in T2 ($p<0.001$) paralleled with an increase in ACE protein levels (200% in T1 and 251% in T2, $p<0.001$). Soleus AGT levels (52% in T1 and 96% in T2, $p<0.05$), ANG II levels (26% in T1 and 46% in T2, $p<0.05$) and VEGF levels (30% in T1 and 60% in T2, $p<0.05$) also were higher in all trained groups. In addition, AT1 receptor protein levels increased after training (39% in T1 and 48% in T2, $p<0.05$). Our data show that SRA participates ET-induced skeletal muscle angiogenesis, which could be associated with regulation of select miRNAs, providing a new target for modulating vascular formation and suggest that miRNA-27a and -27b can be a potential therapeutic target for pathological conditions involving capillary rarefaction.

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Identification of a Novel Prognostic microRNA Signature in Mantle Cell Lymphoma.

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Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma (NHL) that is sensitive to combination chemotherapy, with short remission durations. The clinical course is variable; some patients succumb quickly, while others survive >10 years. MicroRNAs (miRs) are small, non-coding RNAs that regulate gene expression by inhibiting mRNA translation. miRs are useful in the prognostic assessment of tumors, but work to date has only identified 2 miRs involved in MCL prognosis. We hypothesized that a miR signature obtained by comparing miR expression profiles of aggressive NHL with indolent NHL, when applied to a set of MCL cases, may aid in MCL prognosis.miR expression profiles of 20 aggressive and 19 indolent NHL (excluding MCL) were compared to one another to generate a signature based on differential miR expression between the two groups. The most significantly deregulated miRs were then validated on an independent set of 19 aggressive and 25 indolent NHL (excluding MCL). Expression of validated miRs was further tested on a set of 238 MCL samples acquired from 4 separate institutions. Principal component analysis of the miR signature was used to devise a scoring system dividing the MCL cases into separate prognostic groups. This was compared to Ki67 scores to determine prognostic utility of the miR signature. miR expression analysis on the training set of 20 aggressive and 19 indolent NHL yielded 80 significantly deregulated miRs. The 14 most significantly deregulated miRs (FDR<0.02) were analyzed on a validation set of 19 aggressive and 25 indolent NHL, yielding 9 validated miRs. Expression of these 9 miRs was determined on a set of 238 MCL cases. Following principal component analysis, the MCL cases were divided into 3 prognostic groups based on miR expression: a good group (median overall survival (OS):50.2 months), an intermediate group (median OS:37.5 months) and a poor group (median OS:9.4 months) (log rank p<0.0001). Using Ki67 cutoffs of 10 and 30% as reported in the literature on the same sample set was not significant (log rank p=0.5212). We have discovered a miR signature that defines aggressiveness in NHL. This signature shows prognostic value in MCL and is independent of the Ki67 index.

microRNA networks in pancreatic cancer.

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Pancreatic adenocarcinoma represents 90% of all pancreatic cancer cases. Since these tumors are often asymptomatic, they are difficult to detect and usually diagnosed in advanced stages. Pancreatic cancers have a high mortality rate, which is similar to its incidence. The main reason for this high mortality rate is the inefficacy of currently available treatments. In Brazil, pancreatic cancers represent 2% of all cancer types and 4% of all cancer-related deaths. A number of microRNA (miRNA) studies on pancreatic cancer have been published; however, systematic, integrative analyses identifying miRNA gene targets and relevant protein-protein interaction (PPI) networks of genes regulated by miRNAs are lacking. In our study, we performed a meta-analysis of previously published miRNA data in pancreatic adenocarcinoma and normal pancreatic tissues. miRNAs are small RNAs (18-22 nucleotides) located inside "fragile" sites, minimal regions of loss of heterozygosity, common break-points associated with cancer, and minimal regions of amplification. They can regulate gene expression and act as tumor suppressors or oncogenes. For our analysis, we selected seven studies that had available data on primary human pancreatic adenocarcinoma. Altogether, these studies reported 65 microRNAs deregulated in tumors from 465 patients. We integrated miRNA expression data and identified their gene targets using microRNA Data Integration Portal (mirDIP) http://ophid.utoronto.ca/mirDIP. Our goals were to first identify miRNA-regulated targets, and their corresponding PPI networks by mapping gene targets to Interologous Interaction Database (I2D, http://ophid.utoronto.ca/i2d),and potential pathways involved in pancreatic oncogenesis. Identified genes were functionally annotated using publicly available databases (NCBI, UCSC Genome Browser). miRNA:gene and PPI networks were visualized using NAViGaTOR (http://ophid.utoronto.ca/navigator). By integrating data from several studies, we were able to identify PPI networks within relevant pathways mainly regulated by miRNA-181 and miRNA-148 families in pancreatic carcinoma. PPI networks identified genes with roles in signal transduction and cell cycle progression, regulation of metabolism, apoptosis, among other pathways relevant to tumorigenesis. miRNAs and pathways identified in our meta-analysis will be validated in a large, retrospective sample set of pancreatic adenocarcinoma and normal pancreatic tissues. In addition, PPI networks may be used for functional in vitro and in vivo studies to investigate their roles in pancreatic oncogenesis.

microRNA networks in lung cancer.

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Lung cancer is the most common cancer and the main cause of cancer death worldwide, with an incidence in Brazil of over 27,000 new lung cancer cases every year. Despite advances in improved early diagnosis, availability of prognostic and predictive molecular signatures, patient prognosis remains poor and high mortality rates are observed. The development of molecularly-targeted therapies in lung cancer benefits only a small fraction of patients with non-small cell lung cancer (NSCLC), which is the most common histological type of this tumor. Therefore, improving prognostic and predictive signatures and identifying new targets for treatment may lead to increasing patient survival. Recent studies have shown the role of microRNAs (miRNAs) in oncogenesis and their value as prognostic and predictive biomarkers in lung cancer. MiRNAs are small non-coding RNAs (18-22 nucleotides) that have diverse functions, including the regulation of development, cellular differentiation, proliferation and apoptosis. Since miRNAs can function as tumor suppressors or oncogenes they may play a key role in the initiation and progression of cancer. While deregulated miRNA expression in lung cancer versus normal lung tissues have been reported, systematic and integrative analyses that would identify miRNA targets, relevant protein-protein interaction (PPI) networks and affected pathways are lacking. Thus, we performed a meta-analysis of 19 previously published studies, which had available miRNA expression data on primary human NSCLC. Combined, these studies reported 103 microRNAs deregulated in 901 tumors compared to 776 normal lung tissues. Of the 103 deregulated miRNAs, 20 miRNAs were identified by at least 3 studies: miR-21, miR-30 family (miR-30a, b, c, d), miR-31, miR-126, miR-138, miR-139, miR-141, miR-143, miR-145, miR-155, miR-182, miR-200b, miR-200c, miR-205, miR-210, miR-429 and miR-451. Next, we identified predicted miRNA targets (for all 103 miRNAs) using microRNA Data Integration Portal (mirDIP) (http://ophid.utoronto.ca/mirDIP). We then integrated miRNA expression data and predicted miRNA targets using Interologous Interaction Database (I2D) (http://ophid.utoronto.ca/i2d). Our main goal was to identify PPI networks of deregulated miRNAs and miRNA-regulated genes in NSCLC. miRNA:gene and PPI networks were visualized using NAViGaTOR (http://ophid.utoronto.ca/navigator). Identified gene targets were functionally annotated using publicly available databases (NCBI, UCSC Genome Browser). By integrating data from several studies, we were able to identify PPI networks and relevant pathways deregulated in NSCLC. PPI networks included proteins with roles in transcriptional control, signal transduction, cell death, among other relevant pathways with roles in tumorigenesis. miRNAs and pathways identified in our meta-analysis will be validated in primary human NSCLC. In addition, functional in vitro and in vivo studies are required to further determine their roles as drivers of lung oncogenesis. Clinical relevance of these studies includes the development of novel, individualized treatment strategies for patients with NSCLC.

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Expression profile of microRNAs in the hemoglobinophaties.

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MicroRNAs (miRNAs) are negative regulators, which suppress the gene functions through translational repression by targeting 3′-UTR in messenger RNAs (mRNAs) of protein-coding genes or by inducing instability of mRNAs. During erythropoiesis, a complex multistep process encompassing proliferation and differentiation of hematopoietic stem cells to mature erythrocytes, numerous miRNAs are induced or repressed. There is an increasing number of studies demonstrating the function of these non-coding RNAs in the pathogenesis and prognosis of erythropoietic disorders, including betha-thalassemia (βT) and sickle cell anemia (SCA). The aim of this study was to analyze the expression profile in BT and SCA patients of the miRNAs 24, 144, 155, 210, 221, 222, 223 and 451, which all of have been previously described in normal erythropoiesis. Reticulocytes were separated from peripheral blood samples of subjects with normal hematological data (n≥8), from untransfused BT IVS-I-6 (T□C) homozygous patients (n=9) and from SCA patients (n=8), without HU therapy. Spherocytosis patient samples were included in the results analysis. Extraction of miRNA, transcription to cDNAs and Real Time PCR were performed to analyze miRNAs expression, using U47 and RNU6B as endogenous controls. MiRNA 451 is coexpressed with miRNA144 in a bicistronic pri-mRNA transcript whose synthesis is directly activated by GATA-1. This study showed that the expression profile of these miRNAs was decreased in BT patients, compared to controls, in agreement with previous studies which demonstrating that miRNA 144/451 deficiency results in erythroid hyperplasia, ineffective erythropoiesis and anemia. In addition to these miRNAs, we also analyzed the expression profile of the miRNAs 24, 155, 210, 221, 222 and 223, which were significantly lower in BT patients ($p<0.05$). The low expression of these miRNAs could result from oxidative processes in thalassemic red blood cells, due to the imbalance in the synthesis of alpha and beta chains that leads to a decrease in hemoglobin production and promotes free iron accumulation. This event possibly contributes to membrane's lipid peroxidation and may cause miRNA degradation by a toxic microenvironment. The expression of miRNA 221 was significantly lower in SCA patients ($p<0.05$), while the miRNAs 223 and 451 were significantly overexpressed in this group, compared to control subjects. Studies showed that several miRNAs were over-represented in the HbAA erythrocytes while others (including miRNAs 144 and 451) were overexpressed in the HbSS erythrocytes. Many others studies demonstrated the functional importance of miRNAs during various stages of erythroid differentiation. Our findings suggest that miRNAs are involved in the reticulocyte terminal differentiation process and their molecular pathways may contribute to the understanding of BT and SCA.

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Downregulation of microRNAs-1, -133 and -145 in gallbladder cancer.

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Gallbladder cancer (GBC) is the most common malignant lesion of the biliary tract and the fifth most common malignant neoplasm of the digestive tract. GBC is an aggressive disease with a poor prognosis and it's often diagnosed in advanced stages. microRNAs (miRNA) are noncoding short RNA molecules that inhibit gene expression post-transcriptionally. miRNA expression profiles may become useful biomarkers for diagnostics, prognosis and prediction of response to treat and it could be a powerful tool for cancer prevention and therapy. An expression profile of miRNAs in GBC was obtained by Dharmacon® platform microarray. Normal tissue and tumors were compared, resulting in differential expression of 108 miRNAs, 68 of which were downregulated in tumor tissues of gallbladder (fold-change $\geq$ 2). Using three separate algorithms: PicTar, TargetScan, and miRanda were identified miRNAs as putative regulators of overexpressed genes in GBC (VEGF, ErbB3 and CTGF) including miR-1, miR-29c, miR-126, miR-133a, miR-133-b, miR-143, miR-145, and miR-148. To quantify the expression of miRNAs was used TaqMan® miRNA assays qRT-PCR in fresh frozen tissue. The results shows that miR-1, miR-133a, miR-133b and miR-145 are downregulated in tumour ($p<0.005$), suggesting an important role of these miRNAs in the gallbladder carcinogenesis. However, further studies are necessary to evaluate the in vitro effect of these miRNAs in gallbladder cell lines.

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Correlated expression patterns of miRNAs and target genes during the spermatogenesis of honey bee drones.

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Spermatogenesis is a biological process that comprises the development of the germ cells up to spermatozoa. The whole process can be subdivided into three main phases: spermatogonial proliferation, meiosis of spermatocytes and spermiogenesis of haploid spermatids. These phases are marked by several events of apoptosis, autophagy and cell proliferation/differentiation, which are regulated by gene expression. Recently, studies in mammals and Drosophila spermatogenesis show that post-transcriptional control of gene expression is fine tuning of small non-coding RNAs, miRNAs. Up to now, the presence of miRNAs in honey bee drone testes and their putative roles in apoptosis and during germline cell differentiation were not investigated. Based on this, using the information from honeybee miRNA data bank we selected miRNAs involved with the biological processes of interest. We determined the expression profiles of four miRNAs (miR-7, miR-14, miR-184 and bantam) and their putative target genes in drone testes during larval, pupal and adult stages. We used RNAhybrid tool to predicted miRNA target genes and qRT-PCR analysis to assess the expression of these miRNAs and their putative targets (apoptosis inhibitors: bruce; apoptosis activators: caspase 4, jazigo, liz and ark). Our results demonstrate that during mitotic development of spermatogonia in the 4th larval instar, high levels of miR-184 and miR-14, and low levels of apoptotic genes are observed. The abundance of miR-184, miR-14 and miR-7 decrease when spermatocytes are differentiating in the 5th larval instar and this is followed by the formation of spermatids and mature spermatozoa during the first half of the pupal stage. The levels of apoptotic genes transcripts increase during this period, except bruce, which showed decreased mRNA levels. In the newly-emerged drones, we observed a high level of miR-184, miR-14 and miR-7 and low levels of apoptotic genes. Taken together, these results suggest that miR-184 and miR-14 are involved in spermatogenesis. This new insight into the study of drones spermatogenesis reveal gene networks regulating spermatozoa formation and differentiation in insects.

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The effects of violacein in the expression of microRNAs that regulate genes involved in the epigenetic machinery and signaling pathways of chronic myeloid leukemia.

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DNA methylation in mammals is an important epigenetic modification, playing an essential role in the silencing of repetitive DNA, in genomic imprinting and, in females, the establishment of X chromosome inactivation. DNMT3B, a DNA methyltranferase, has an important role in de novo methylation. This may lead to important alterations in chromatin conformation, therefore inhibiting gene transcription. Changes in the expression patterns of DNMT3B and, consequently, in DNA methylation, are found in several types of tumors, including leukemia. Alterations in the expression of miRNAs can contribute to the deregulation of DNMT3B, and recent studies have reported that the re-introduction of miR-29b in leukemia cells is capable of silencing DNMT3B, leading to the re-expression of several tumor suppressor genes. Additionally, the expression of ABL and BCR-ABL, two tyrosine kinases, is frequently reported to be upregulated in leukemias, while miR-203, a miRNA that regulates their expression, is usually found downregulated in these cells. Violacein, a pigment produced by Chromobacterium violaceum, a bacteria found in the Amazon region, has shown a potential antitumor effect in leukemia cells, but the pathways involved in this activity is not completely understood. Thus, the aim of this work was to observe the effects of violacein in the expression of DNMT3B, ABL, BCR-ABL, miR-29 family and miR-203 genes in K562, a chronic myeloid leukemia (CML) cell line. It was observed a downregulation in the expression of DNMT3B concurrently with an upregulation of miR-29 family after violacein treatment. The expression of ABL was also decreased after violacein treatment, while an increase in miR-203 expression was found. Although an upregulation of the BCR-ABL mRNA expression occurred after treatment, BCR-ABL protein levels were decreased. These data show that violacein is capable of downregulating the expression of genes involved in the epigenetic machinery and in the regulation of signaling pathways associated with leukemogenesis, concomitantly with an increase in the expression of regulatory miRNAs. In conclusion, our results indicate that this compound may target key pathways known to be altered in leukemia cells, which is encouraging when considering its possible use in combined therapy of CML.

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Differential expression profile of microRNA in anencephaly.

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Anencephaly is one of the most severe forms of neural tube defects (NTDs), and is characterized by full or partial absence of skull, brain and scalp. However, some rudimentary cerebrovascular tissue is usually identifiable. Exencephaly, absence of cranium with exposure and posterior degeneration of nerve tissue, is not clinically differentiable from anencephaly in the older fetuses. MicroRNA has been shown to play an important role in development of the central nervous system; their role in human NTDs is poorly understood. We report a series of microRNAs differentially expressed in anencephaly in comparison to normal fetal frontal cortex and depict putative target genes. Four surgical specimens were obtained from fetuses with anencephaly and six normal frontal cortex tissues were obtained from fetal autopsy. Total RNA was extracted by Trizol according to manufacturer's instructions, with an additional overnight precipitation step at -20°C with isopropanol. Five hundred micrograms of RNA were labeled with the 3DNA FlashTag Biotin HSR (Genisphere) and hybridized to GeneChip miRNA Arrays (Affymetrix). Data was acquired using a GeneChip Scanner 3000 7G (Affymetrix). The data processing was done in R environment (www.r-project.org) and the packages Affy and RankProd from Bioconductor were used for microRNA expression profile analysis. MicroRNAs were considered differentially expressed when the statistical significance was $p \leq 0.01$. The prediction of potential targets of selected microRNAs was done using the miRGen Targets and miR-Ontology Database softwares. A total of 128 microRNAs were differentially expressed in anencephaly. Of these, 61 were upregulated and 67 downregulated. The miR-199b-5p, miR-199b-3p, miR-375, miR-455-3p, miR-449a and 886-3p were among upregulated microRNAs while miR-128, miR-137, miR-330-3p, miR-383, miR-650 and miR-770-5p among downregulated ones. Target genes of these microRNAs include ENAH (miRs-128, 137, 330-3p, 650, 199b-5p and 375), NPTX1 (miRs-128, 330-3p, 650, 199b-3p/5p, 455-3p, 449a, and 886-3p), DVL2 (miR-128, 137, 199b-5p and 449a), MTHFR (all except miRs-199b-5p, 455-3p and 886-3p) and PAFAH1B1 (miRs-330-3p, 383, 199b-3p/5p, 375, and 455-3p) which are known to be involved in neural tube defects, cell differentiation, neuronal migration, nervous system development and Wnt receptor signaling pathway. Result of our study may help understand the potential role of individual miRNA in anencephaly.

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MicroRNAs associated with melanoma progression.

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miRNAs are non-coding RNAs that bind to mRNA targets preventing their translation. It is predicted that over 30% of mRNAs are regulated by miRNAs. Therefore, these molecules are considered essential to many biological responses, such as cell proliferation, apoptosis and stress responsiveness. Loss or gain of miRNAs' function contributes to development of many diseases, including cancer. Melanoma rises from malignant transformation of melanocytes. Despite being rare, it is responsible for the greatest number of deaths promoted by all skin cancers. One reason for this scenario is that metastatic melanoma is usually incurable. To determine the role of miRNAs in melanoma progression, the expression of several miRNAs were evaluated in a murine model that mimics different stages of the genesis of this tumor. In that model, several melanoma cell lines were obtained after submitting melan-a melanocytes to sustained stress conditions. Among miRNAs in which expression were altered, miR-138 has decreased expression in intermediate phases of melanoma genesis and was selected for further investigation. It was verified that mRNAs of p53, Ezh2, MyoD and Sirt-1 are putative targets of miR-138. Interestingly, these mRNAs also have inverse expression of miR-138 in the distinct lineages of this model. Luciferase assay was done to confirm if miR-138 directly regulate such mRNAs. Superexpression of miR-138 in the cell line that has lower levels of this miRNA was also induced. Besides miR-138, we also demonstrated that miR-340-5p, miR-678 and miR-330 have their expression altered during melanoma genesis. These miRNAs were selected as putative targets of Cyclin D1, Cdk4 and p21 mRNA, respectively. Moreover, it was verified that miR-340-5p, miR-678 and miR-330 have inverse expression of Cyclin D1, Cdk4 and p21 mRNAs, consecutively, in the different lineages that represent melanoma progression. The targets of these miRNAs are key components in pathways frequently disrupted in cancer, like cell cycle and apoptosis control, epithelial to mesenchymal transition regulation, and differentiation process. Thus, these miRNAs represent strong candidates to therapeutic targets. The exploration of their roles can contribute to improve the comprehension about melanoma progression and elucidate new ways of interventions against cancer.

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Potential Growth Regulation of miR-100 in Childhood Acute Lymphoblastic Leukemia.

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MicroRNAs are small non-coding endogenous RNAs that function predominantly as sequence-targeted modifiers of gene expression through translational repression. Disregulation ofthe miR-100 has been shown in several neoplasias including ovarian, lung, medulloblastoma, pancreatic, liver and prostate cancer. Additionally, it has also been implicated in the regulation of some important oncogenes such as IGF2and PLK1. Recently, our group described the association of miR-100 altered expression and clinical features, such aslow white blood cell count at diagnosis and t(12;21),in pediatric acute lymphoblastic leukemia(ALL);however in vitro effects of this microRNA in ALL cell lines hasnot been tested. In this context, the present study aimed to evaluate the in vitro effects of miR-100 forced-expression in B and T ALL cell lines. Pre-microRNA miR-100 and negative control were transfected into Jurkat (T-ALL) and ReH (B-ALL) cell lines using Lipofectamine 2000 at a final concentration of 100 nM. Cell proliferation by XTT kit, colony formation assay in methocult® medium and apoptosis by anexin V were assessed. A small but significant decrease in proliferation ofReH cell line after 120 hours after transfection and a decreaseinnumber of colony formation in Jurkatwas observed. The apoptosis index was not altered after transfection. These findings suggest a potential negative effect of miR-100 in ALL cell growth and support the association between lower expression levels and poorer prognosis in childhood ALL found in patients samples.

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MicroRNAs targets prediction in complex diseases.

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The fact that the majority of diseases are related to genetics factors is already known. However, different from autossomal dominant diseases that are related to a single gene, complex diseases involve several genes and present a challenge for the understanding of these genetic factors. Recent genetic discoveries have identified new genes involved in many complex diseases such as psychiatric disorders. But how these genes are regulated and related to many behavioral disorders is far from being completely elucidated. MicroRNAs are members of a family of non-coding RNAs of ~22nt participating in the process of gene expression through events of negative regulation. These microRNAs can hybridize to the 3' UTR of mRNAs to direct their post-transcriptional repression through translation inhibition. It is estimated that microRNAs regulate ~60% of coding-protein genes through this mechanism and that 1-2% of all human genes are genes of microRNAs. Currently studies show that approximately one-third of the microRNAs are expressed in the brain, where they have been shown to be involved in maintaining the brain function and are closely related to affecting synaptosomal complex, neuronal differentiation and others effects. These events are exactly the same interrupted at several psychiatric disorders. There are many computational tools for microRNA target prediction in animals. Studies with microRNAs and their targets can help to understand the involvement of microRNAs in these diseases. We are performing a computational prediction of microRNA targets in genes related to schizophrenia. The predictions are analyzed together with gene expression data from brains of individuals affected with schizophrenia and controls.

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High-throughput miRNA expression profiling to predict rectal cancer treatment response.

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Neoadjuvant chemoradiation therapy (CRT) may lead to complete tumor regression in a significant proportion of patients with distal rectal cancer. In these patients, alternative surgical procedures to total mesorectal excision have been considered in an effort to avoid unnecessary postoperative morbidity and functional disorders. Still, patient selection is very difficult and based on rather imprecise and subjective radiological and clinical findings. In this setting, identification of molecular markers capable of predicting complete tumor response to neoadjuvant CRT would aid in the selection of patients that are most likely to benefit from CRT and who are potential candidates to these alternative treatment strategies. The purpose of this study was to compare microRNA expression patterns among patients with complete and incomplete tumor regression to neoadjuvant CRT. Pre-treatment biopsies from 33 patients with cT2-4N0-2M0 distal rectal adenocarcinoma were prospectively collected. Total RNA was extracted, enriched for small RNAs and used for microRNA expression analysis on SOLID4 sequencer. Sequences were mapped against the human genome reference sequence using LifeScope. Overall, 11 patients with complete tumor regression were compared to 22 patients with incomplete tumor regression. An average of 30 million reads was generated for each patient. miRNAs were differently expressed in the two groups, among them miR-191, miR-30b and miR-30d. These miRNAs have already been associated with worse prognosis in different tumor types, and might also be involved with treatment response in rectal cancer patients.

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Developmental genes network of haploid *Apis mellifera* male embryo.

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In honeybees, *Apis mellifera*, fertilized eggs development result in females (2n) and unfertilized eggs in males (n). However, the activation of the egg that results in the haploid zygote formation and subsequent male development is still a mystery. Genes and regulatory pathways of development, most expressed in embryogenesis, are conserved among various insects, however is unknown in honeybees. In the transition from maternal to zygotic phase, during the early phases of development, a subset of maternal mRNAs is degraded and then the zygote genome is activated. This zygotic transcription leads to the production of specific proteins and microRNAs. The miRNA, essential to gene expression regulation has been described in D. melanogaster at various stages of development, and linked to the regulation of developmental processes. miRNAs are clearly involved in fine tuning genomic responses as well as in systemic responses to environmental changes. Herein, we investigated the honeybee transcriptome (mRNA and miRNA) during early phases of haploid embryonic development. By means of Illumina deep-sequencing of small RNA and mRNA extracted from haploid eggs of 0-6h of development we constructed two libraries. Searching the 18 edition of MIRBASE that hosts 167 A. mellifera miRNA, we found that 160 are represented in our libraries, been the more expressed, ame-mir-3743 and ame-mir-3744, described as honey bee specific. We used RNAhybrid software to search for miRNA-binding sites to 3'UTR from a set of 46 up- or down-regulated genes linked to embryonic development, using seed pairing (1-8) and -20 of free energy as criteria. Our data revealed an interacting network with 60 miRNAs and 46 targets. The most connected miRNA was ame-miR-3776 (7 targets) and the most connected genes were GB10275 and GB10122 (10 miRNAs). Among all miRNAs, ame-mir-184, described as an important regulator of embryogenesis, is one the most expressed in our library (278 reads). Looking to the codifying gene we found vg mRNA (vitellogenin, GB13999) a gene linked to development and reproduction that is coherently down regulated in our library, and could be targeted by 8 miRNAs. Growing evidences suggest that ncRNAs play an important role in transcriptional and post-transcriptional regulation and that the understanding of their function will give us new possibilities to get some light to the intricate network responsible for embryonic development.

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MicroRNA-208a is involved in regulation of cardiac hypertrophy induced by aerobic training in rats.

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MicroRNAs (miRNA) are small non coding RNAs that regulate post-transcriptional expression of their target genes. The miRNA-208a is exclusively expressed in heart and regulates gene expression of slow myosin heavy chain (βMHC). We investigated the role of miRNA-208a in cardiac hypertrophy (CH) induced by aerobic exercise training (TF). Female Wistar Rats (n=21) were randomized into three groups: sedentary (S), Trained 1 (T1-moderate volume training): swimming sessions of 60min, 5 days/week, during 10 weeks, with caudal dumbbells weighing 5% of body weight. Trained 2 (T2-high volume training): similar to T1 until 8th week, on the 9th week the rats swan twice/day and on the 10th week 3 times/day. Were assessed: 349 cardiac microRNAs by microRNA Array, heart rate (HR) e blood pressure (BP) by caudal pletismography, VO2max by ergoespirometry and ventricular function and CH by echocardiography, LV/BW ratio (mg/g) and cardiomyocyte diameter, gene expression of pathological cardiac markers (ANF, skeletal α-actin, α/β-MHC) and miRNA-208a by Real-time PCR. $P<0.05$ was accepted as statistically significant. TF decreased HR and did not change the BP. VO2max increased (11% in T1 and 15% in T2). LV/BW ratio increased 13% in T1 and 28% in T2 and cardiomyocyte diameter increased 20% in T1 and 31% in T2. There were not alterations in CH pathological markers gene expression. There was no difference among groups in cardiac systolic function, however the ratio E/A increased 17% to T2. IVRT decreased 11% to T2, DT decreased 17% to T1 and 14% to T2. MPI decreased 30% to T1 and 25% to T2. MiRNA-208a expression decreased 62% (vs.S) and 46% (vs.T1) to T2 concomitantly with increase of α/β-MHC ratio, inversely to that observed in pathological CH. Aerobic Training promoted physiological CH, improved diastolic function and decreased MiRNA-208a and β-MHC gene expression. The exercise-induced inhibition of miR-208a can be a non-pharmacological therapeutic strategy to repress the β-MHC gene expression in pathological CH.

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miR-15a/16-1 influence Bcl-2 expression in keratocystic odontogenic tumor.

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The keratocystic odontogenic tumor (KCOT) is a benign destructive recurrent odontogenic cystic neoplasm. MicroRNAs (miRNAs) miR-15a and miR-16-1 are small non-protein coding RNAs that function as negative regulators of the anti-apoptotic gene BCL2 at the post-transcriptional level. Notably high Bcl-2 immunoexpression is found in the epithelial lining of KCOTs while loss of Bcl-2 immunopositive cells are observed in marsupialized cysts. The purpose of the present study was to investigate whether miR-15a and miR-16-1 transcription is altered in the KCOT and if it is associated with BCL2 gene expression in such lesions. By using qRT-PCR, we found miR-15a and/or miR-16-1 downregulation in the majority of KCOT samples (24/28). We also found higher BCL2 mRNA expression in 19 out of 20 KCOTs frozen samples through qRT-PCR. Additionally, moderate to high Bcl-2 imunopositivity was found in the basal layer cells in 16 out of 18 paraffin embedded KCOT (median: 42.6%). In vitro over-expression of miR-15a/16-1 in human KCOT-1 primary cell culture resulted in a decrease of Bcl-2 protein expression. Furthermore, five KCOTs collected before (primary tumor) and after the marsupialization procedure (marsupialized turmor) exhibited increased levels of miR-15a expression after the marsupialization procedure ($p = 0.04$). Our results strongly suggest that KCOT neoplastic cells present an anti-apoptotic profile to which a lower miR-15a/16-1 expression might be related. Additionally, we demonstrated that miRNA expression increases after marsupialization, implicating an aetiological and therapeutic role of miRNAs in KCOT.

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MicroRNA pattern discriminates between early onset familial and sporadic breast cancer cases.

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Breast cancer in patients under 35 is uncommon, occurring in only 2–10% of the cases in western countries, but this value may differ among ethnic groups. Breast cancer in young woman has been correlated to poor survival and aggressive features. Family history was observed in 10%-37% of these patients but only 10%-40% of these cases displayed mutations in BRCA1/2 genes, and a lower proportion occurs in cases without familial history (3-10%). However, the genetic basis of early onset breast cancer with or without familial history, non carriers of BRCA1/BRCA2 mutations is not well elucidated but aggressiveness may be derived from specific biological characteristics. The deregulation by microRNAs has recently emerged as a major determinant of tumorigenesis. Because miRNAs function by targeting functionally important protein-coding genes, it is of outstanding interest to identify miRNAs involved in the molecular mechanism underlying aggressiveness in tumors of young patients that might represent biomarkers and therapeutic targets. We aimed to identify a miRNAs expression signature that could discriminate between familial and sporadic breast cancer derived from young patients non carriers of BRCA1/2 mutations. Methodology: Thirty-five patients were selected: 9 with familial history of breast and ovarian suggestive of hereditary condition according to NCCN criteria and 26 without familial history, non carriers of BRCA1/BRCA2 mutations. The determination of microRNA expression network between those 2 groups was performed by TaqMan microRNA Assay (Applied Biosystems). Data were normalized using endogenous miRNA presented in each array. The normalization between samples was performed by limma library R version 2.13. Statistical comparisons were done using Significance Analysis of Microarrays (SAM) test with adjusted FDR (0%). The validation of the cases distribution for familial and sporadic groups was performed by cross-validation. We found that 9 miRNAs were differently expressed between the 2 groups (familial and non familial). Among them, 2 miRs (miR-381 and miR-455-3p) were down regulated whereas 7 miRs (miR- 124, 210, 98, 486-3p, 501-5p, 660, 874) were up regulated in tumors related to the familial group as compared to sporadic tumors. Our findings suggested that discrete differences of microRNA expression patterns discriminate between tumors from familial or sporadic cases with 75% and 85% of accuracy respectively. Based on the literature and target prediction´s data bases, most of these miRNAs targets are related to focal adhesion, insulin signaling pathway, actin cytoskeleton regulation and MAPK signaling pathway. To our knowledge, this is the first evidence of miRNA expression in tumors of early onset breast cancer patients, non carriers of BRCA1/2 mutation, providing insights that may lead to the comprehension of the mechanisms of carcinogenesis in these patients.
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Chilean propolis modulates the miR-126 expression in aortic tissue of hypercholesterolemic C57BL/6 mice.

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Numerous studies have shown that polyphenols from propolis can modulate different atherosclerosis risk factors, like inflammation, angiogenesis, lipids, oxidative stress, among others, but there is little evidence about the molecular mechanisms involved on it. Current evidence, has demonstrated that polyphenols at nutritional doses can modulate the expression of microRNAs in the liver, but no exist evidence in vascular tissue. Also, there have not antecedents of how polyphenols from propolis can modulate this expression. Thus, the objective of the study was to evaluate the effect of polyphenols from propolis on the expression of mir-126 and mir-155 in aortic tissue of C57BL/6 mice. Fifteen males C57BL/6 mice were divided into three groups (n=5 mice per group): a control group fed with a normal diet (C group), other one fed with atherogenic diet (HC group) and another one fed with atherogenic diet supplemented with propolis 40mg/kg/day (P group) during 16 weeks. RNA from aortic tissue was extracted and the expression of mir-126 and mir-155 were studied by real time PCR, using three reference genes (Rpl4, Rpl13a and Rps29). The statistical analysis was done with the software REST 2009. The mir-126 was down-regulated in HC group compared with C group ($p=0.023$). Expression differences were not observed between the C group and P group ($p=0.937$). The expression of mir-155 was not significantly different among the studied groups ($p>0.05$). The down regulated expression of mir-126 has been related with many pathologic conditions such as cancer and coronary artery disease and our results in the HC group are consistent with this observation. In summary, this study shown that polyphenols from propolis modified the expression of mir-126, suggesting a possible role in cardiovascular risk prevention.

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MicroRNA 499 is up regulated in red muscle of adults pacu (*Piaractus mesopotamicus*).

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Recent studies have demonstrated that a class of small nuclear RNAs, the microRNAs, are involved in controlling the phenotype of slow or fast skeletal muscles, including the microRNAsmiR -499, -208b, -1, -133a, -133b e -206. The aim of this study was to evaluate the expression of the microRNAs miR -499, -208b, -1, -133a,-133b and -206 in red and white muscle of pacu. Adults fish (n = 6) were anesthetized, euthanized and samples of red and white muscle were frozen in liquid nitrogen for the gene expression analysis of the microRNAs by RT-qPCR. For statistical analysis, a variance of 5% was considered statistically significant ($p < 0.05$).Our results showed a down regulation of miR -1 and -133a and a up regulation of miR -499. Our major finding is that, as described in human, mouse and rats, miR 499 is up regulated in red muscle of adults pacu (Piaractus mesopotamicus) and may be involved in the maintenance of the slow muscle phenotype in fish.

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Changes in the microRNAs expression profile of fibroblasts associated with breast cancer compared with those obtained from normal breast tissue.

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Cancer-associated fibroblasts (CAFs) contribute to tumor growth and progression in breast cancer. CAFs are functional and phenotypically distinct from normal breast tissue (NAFs). Epigenetic modifications and post-transcriptional gene regulation by microRNAs may contribute to the phenotype of CAFs. There are no current studies characterizing the involvement of expression of miRNAs of CAFs in breast cancer. We aimed to identify the changes in microRNAsexpression profile in fibroblasts associated with breast cancer as compared to those observed in fibroblasts obtained from samples of mammoplasty obtained from São Paulo Hospital. CAFs were obtained from tissue samples diagnosed with primary ductal infiltrative breast cancer from patients operated at Hospital PérolaByington.After establishment of primary culture of breast carcinoma (n= 04) and normal breast (n= 05) fibroblasts,whichwere characterized by vimentin, alpha-actin and S100A4 presence and total absence of CD31 and pan-cytokeratin expression determinatedby Immunohistochemistry. The total RNAs from cell culture were extracted and purified using the Mirvana kit and their quality evaluated by Bioanalyzer. After PCR TaqMan MicroRNA assay-system-array, statistical analysis by Test t Student e Mann-Whitney show five microRNAs differentially expressed: h_miR_655, h_miR_383, h_miR_129_3p, h_miR_15b, h_miR_489 (FDR 0% and p =0,05).The combined miRNAsexpression analysis and their RNA targets to be determinedby microarray in CAFs may contribute to understanding the microenvironment influence on breast carcinoma cells.

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In silico identification of potential miRNAs regulating oxidative stress related genes in aging and longevity.

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The oxidative stress hypothesis of aging suggests that accumulation of oxidative damage during lifespan may be related to loss of cellular functions, a process normally associated with senescence. Epigenetic regulation of gene expression via microRNAs (miRNA) is an important mechanism linked to basic cellular process such as proliferation, apoptosis and differentiation, which are intrinsically related to aging. We aimed to identify miRNAspotentially involved in the regulation of oxidative stress related genes using an in silico selection, based on specific prediction and conservation parameters. Nine genes previously found to be differentially expressed in peripheral blood in elderly and long-lived individualswere selected(PRNP, CCL5, BNIP3, GPX7, EPX, SIRT2, SRXN1, GPX1 and DGKK) and miRNAs predicted to regulate these genes were investigated in four databases (miRTar, TargetScan, micrRNA.org and miRDB). Selection criteria were as follows: only miRNAs that bind to 3'UTR were selected; miRNAs appearing only in one database were excluded; and miRNAs with prediction mirSVR score above -0.90 and PhastCons conservation score below 0.53 were excluded. From an initial pool of 343 miRNAs predicted to regulate the aforementioned genes, 14 passed the selection criteria (miR-410, -376c, -340-5p, -146b-5p, -33a-5p, -320a, -145-5p, -411-5p, -202-3p, -1294, -513a-5p, -103b, -1275, -22-3p). The selectedmiRNAs regulates 7 out of the 9 candidate genes (GPX1 and DGKK were not predicted), and each miRNA regulates only one gene. Additional work is being conducted to verify the level of expression of these miRNAs in peripheral blood of young and elderly individuals and validate the in silico analyses. In addition, functional interference studies should be performed to validate the regulation of these genes by the identified miRNAs. The present study proposes a strategy for the selection ofmiRNA potentially involved in the regulation of oxidative stress related genes in aging and longevity, based only in an in silico investigation. The present strategy might be used to identify miRNAs using the differentially expressed genes as start points to elucidate the molecular basis of other biological process.

MicroRNA prediction and expression in *Carica papaya*.

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MicroRNAs (miRNAs) have been shown to play an important gene regulatory role during plant development. They have been associated with multiple biological processes, acting by degrading targeted mRNAs or repressing mRNA translation. To date, a total of 9,274 plant miRNAs are present in the Plant miRNAs Database (PMRD), but only two, miR162a and miR403, are for papaya (Carica papaya). Carica papaya is cultivated world-wide in tropical and subtropical climates, mainly for its fruit. Brazil is one of the largest papaya producers in the world and the state of Espirito Santo is the main producer in the country. Identification of new miRNAs in papaya is important to identify and understand tissue-regulated genes associated with defense, abiotic and biotic stress, and plant development. In this study, known plant miRNAs sequences in Genomic Survey Sequence (GSS) and Expressed Sequence Tag (EST) databases were used to search for potential miRNAs in papaya. A total of six potential miRNAs (MIR171, MIR390, MIR399, MIR535, MIR854 and MIRf10987-akr), representing six gene families, were identified. Secondary structures were predicted using the mfold-3.2 program. Additionally, TaqMan MicroRNA assays (Applied Biosystems) were used to quantify levels of mature miRNAs (miR390a, miR535, miR399f). In conclusion, new miRNAs in Carica papaya were successfully detected and characterized.

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miR-210 is downregulated in Burkitt's lymphoma cells treated with panobinostat, a novel pan-HDAC inhibitor.

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The mechanisms underlying Burkitt lymphoma (BL) chemoresistance and how it can be circumvented remain undetermined. The HDAC inhibitor, panobinostat (LBH589), achieves potent inhibition of all HDAC enzymes implicated in cancer and has demonstrated potent antitumor activity in preclinical models and promising clinical efficacy in cancer patients. Recent findings indicate that HDACi repress angiogenesis, a process essential for tumor metabolism and progression, through hypoxia-inducible factors (HIF) which are the master regulator of cellular adaptation to hypoxia. Recently, microRNAs (miRNA) have emerged as a new class of genes regulated by HIFs in response to hypoxia, of which miR-210 is the most consistent and predominantly upregulated miRNA. Since the discovery of biomarkers as determinants for optimal treatment response and outcome, miRNAs have thus far shown much promise as credible biomarkers. Here we evaluate the expression of HIF1α and mir-210 after the combined treatment of HDACi, LBH589, with Cisplatin (CDDP) or Etoposide (VP-16) in well characterized BL cell lines. Apoptosis and mitochondrial membrane potential (MMP) were accessed by flow cytometry using Annexin-V/PI and DiOC6, respectively. Apoptosiss was enhanced by the combined treatments (Panobinostat/CDDP and Panobinostat/VP-16), when compared with single treatment. The combination of Panobinostat and VP-16 induced apoptosis in 59,9% of Raji cells compared to 20,4% for Panobinostat alone and 33.3% for VP-16 0.3 µM alone resulting in synergistic R value of 0.77. Moreover, in Daudi cell line we observed an additive effects (R=1.1) of both combinations. The apoptosis effects were followed by MMP decreased levels in a dose-dependent manner. The apoptotic profile was correlated with HIF-1α mRNA degradation and downregulation of mir-210. miRNA expression profile may become useful biomarkers identifying the most appropriate responsive tumor, overcome chemoresistance, achieve durable remission and improving survival of BL patients.

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Preliminary evaluation of selected muscle specific miRNAs in plasma of recreational runners before and after a half-marathon (21 km).

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The health benefits brought by physical exercise are well known. However, knowledge about the molecular mechanisms behind such benefits is lacking. Recent evidences point to circulating miRNAs as important molecules driving tissue-tissue communication. Our hypothesis is that muscle specific miRNAs (myomiRs) are delivered to blood during exercise and play a role in the way an exercised muscle improves health in the whole body. The main goal of this experiment was to identify the levels of three myomiRs in plasma of high aerobically adapted individuals and whether their expression was significantly altered due to a half-marathon run.Plasma was obtained from five recreational runners before and immediately after a half-marathon race. The miRNAs were isolated and then quantified in triplicates by real-time PCR using hydrolysis probes to miR-1, -133a and -206. Normalization of sample variation was made using synthetic miRNAs (miR-54 and -238) from Caenorhabditi selegans as spiked-in controls. The relative changes in expression were calculated using the comparative Cq method, where the mean delta Cq of each group was used for comparison. Statisticalt-test was run for all data and results were considered significant at $P < 0.05$. Only miR-206 was higher after running compared to the levels at rest, where the increase was of 4.3 times. Since research on circulating miRNAs has focused on disease,it is unknown how exercise training changes myomiR expression. This is the first study to verify the levels of miR-1 and -206 in plasma before and after exercise in humans. Baggish and colleagues (2011) measured in men12 circulating miRNAs, among them, miR-133a, which also didn't change after exercise. Two different studies, Nielsen et al. (2010) and McCarthy and Esser (2007), showed a decrease in miR-1 and -133a levels in muscle biopsies of mice after aerobic training, while miR-206 was unaltered. However the latter also observed an increase in the levels of the corresponding primary-miRNAs, including pri-miR-206 and also that the expression of miR-206 was significantly higher in the soleus than in the plantaris muscle. So far, the miR-206 function in the adult skeletal muscle specially regarding exercise is unknown, however, there are studies suggesting a role in hypertrophy and fiber type switching. Our results raise more questions than answers and one question we believe to be central is if there is any biological relevance of the increase of themyomiR206 in plasma after exercise. And if there is, which and where?

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Relationship between expression levels of miRNAs and histopathologic features of dysplasia in oral leukoplakia.

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Oral leukoplakia (OL) is the most important potentially malignant oral lesion. The grading of dysplasia has been made with base on histopathologic findings, but the molecular significance of each parameter used to grade lesions has not been established. MicroRNAs (miRNAs) are small non-coding RNA molecules whose function is the post-transcriptional regulation or degrading of thousands of messenger RNAs (mRNAs). The aim of the present study was investigate the association of each cytological or histopathological features used to grade oral dysplasia with the expression of three potentially cancer-related miRNAs (miR-21, miR-345 and miR-181). We evaluated the miRNAs expression by qPCR in sixteen cases of OL with different grade of dysplasia, seventeen cases of oral squamous cell carcinoma samples and six samples of normal oral mucosa. All samples of squamous cell carcinoma showed increased expression of the same miRNA. We found high expression of miR-21 and miR-181 in OL with high grade of dysplasia, especially in the cases that presented drop-shaped rete ridges and cellular and nuclear pleomorphism. High expression of miR-345 was observed in OL with hypercromasia and increased number and size of nucleoli. Our study shows that some cytological and histopathological parameters used to grade dysplasia exhibit a different molecular profile. The significance of this data in OL staging and management needs to be further evaluated.

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Swimming training increases cardiac microRNA-126 expression volume dependent in rats: relationship to angiogenesis.

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MicroRNAs (miRNAs) are non-coding short RNAs that repress the translation of their mRNA target. MiRNA-126 is angiogenic and has two validated targets Spred-1 and PI3KR2, negative regulators of angiogenesis by VEGF pathway inhibition. We investigated, for the first time, the role of miRNA-126 on cardiac angiogenesis induced by swimming training. Female Wistar rats were assigned into 3 groups: sedentary (S), Training 1 (T1, moderate volume) and Training 2 (T2, high volume). MiRNA and PI3KR2 gene expression analysis were performed by real-time PCR. We assessed: markers of training, the cardiac capillary/fiber ratio, protein expression of VEGF, Spred-1, Raf-1 / ERK1/2, PI3K/Akt/eNOS. Both training regimens were efficient in promoting metabolic, morphological and hemodynamic adaptations. The cardiac capillary/fiber ratio increased in Groups T1 (1.34±0.17 a.u.) and T2 (1.71±0.15 a.u.) compared with Group S (0.85±0.10 a.u.). VEGF protein expression was increased 42% in T1 and 108 % in T2. Cardiac miRNA-126 expression increased 26% (T1) and 42% (T2) compared with S, correlated with angiogenesis. The miRNA-126 target Spred-1 protein level decreased 41% (T1) and 39% (T2), which consequently favored an increase in angiogenic signaling pathway Raf-1 / ERK1/2. On the other hand, the gene expression of PI3KR2, the other miRNA-126 target, was reduced 39% (T1) and 78% (T2) and there were an increase in protein expression of components of PI3K/Akt/eNOS signaling pathway in the trained groups. This study showed the involvement of miRNA-126 in the regulation of exercise-induced cardiac angiogenesis, by indirect regulation of the VEGF pathway and direct regulation of its targets (Spred-1 and PI3KR2) that converged in an increase in the VEGF signaling and hence in angiogenic pathways, such as MAPK and PI3K/Akt/eNOS.

Maternal-to-zygotic transition in female embryonic development of *Apis mellifera*.

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Mechanisms that control gene expression operate in many levels: before and after transcription, and after translation. In 1993, a novel class of post-transcriptional regulatory molecules was described: microRNAs. microRNAs (miRNAs) are non-coding RNAs that have 20-23 nucleotides in length after being processed. Mature miRNA molecule joins RISC complex and conducts negative regulation of targeted gene expression. One of miRNAs functions is regulation of maternal-to-zygotic transition in early insect embryonic development. Mechanisms involved in regulation of maternal-to-zygotic transition in honeybee embryonic development are still unknown. Thus, using *Apis mellifera* as a model for embryogenesis, our goals are: 1) Identify maternal miRNAs and mRNAs. 2) Characterize miRNAs: target interactions that modulate maternal products clearance and activation of zygotic genome. For this, mRNAs and small RNAs libraries were built using RNA extracted from five phases of A. mellifera embryonic development. In this study we present: 1) characterization of miRNAs and mRNAs libraries; 2) suggestion of maternal coding genes (deposited during oogenesis); 3) target prediction for expressed miRNAs in A. mellifera embryonic development, based on miRNAs and mRNAs expression profiles and miRNAs binding-sites in mRNAs 3' UTR.

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Investigating the role of microRNA regulation in the development of focal cortical dysplasia.

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MicroRNAs (miRNAs) are small noncoding RNAs which regulate post-transcriptional gene expression. Focal cortical dysplasia (FCD) is a malformation of cortical development which affects up to 36% of patients with drug-resistant epilepsy. The objective of the present work is to investigate a possible role of miRNA regulation in the etiology of FCD. We used brain tissue obtained after surgery for the treatment of medically refractory seizures from nine patients with FCD (four patients with FCD type IIa and five patients with FCD type IIb). In addition, we used cortical tissue from autopsy as controls (n=5). Total RNA was isolated with RecoverAllTM kit (Ambion) and RNA integrity was assessed by Agilent RNA Pico Chip Kit and Bio-Analyzer 2100.MiRNA expression profile was assessed by Affymetrix GeneChip platform miRNA array. Background correction, summarization and normalization were performed by RMA function. MiRNA expression was analyzed using RankProd (FDR $p < 0.05$). Our preliminary analysis identified 23 differentially expressed miRNAs when patients and control group were compared. Furthermore, when FCD type IIa and FCD type IIb groups were compared we found six differentially expressed miRNA types. Among them we observed a significant down-regulation of several elements belonging to the miR-17~92 cluster. This cluster is known to contribute to transcriptional regulation of stem cell differentiation, aging, as well as fine-tuning of pathways involved in neuronal differentiation. Thus, our results clearly show that neurodevelopment pathways are indeed involved in the pathophysiology of FCD. In addition, we identified a different miRNA expression signature in different FCD histological subtypes.

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miR-16 and miR-125b expression in normal and tumoral prostatic cells treated with finasteride.

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Studies have shown that some microRNAs regulate cell proliferation and apoptosis processes that are important in cancer formation. Furthermore, it's well known that many microRNAs are differentially expressed and deregulated in prostate cancer, acting as oncogenes or tumor suppressors. Finasteride, an inhibitor of type 2 5-α reductase, was approved by the American Urological Association to be used in prostate cancer chemoprevention, but its prescription is still controversial between urologists. Considering the importance of microRNAs effects on prostate cancer progression and the actual investigations about the safety of using finasteride in prostate cancer chemoprevention, the objectives of this study were to evaluate miR-16 (tumor suppressor gene) and miR-125b (oncogene regulated by androgen) expression in normal human prostatic epithelial cells (RWPE-1), in androgen dependent prostatic cancer cells (LNCaP) and in androgen independent prostatic cancer cells (PC3) treated with finasteride. During the treatment, cells were cultivated in specific media supplemented with 10 μM finasteride (Sigma-CO™, Saint Louis, MO, USA) dissolved in DMSO (0.1% final concentration). Cells were plated in triplicate at 4 x 105/cm2 and after 5 days of finasteride exposure, total RNA was isolated using TRizol (Invitrogen). Control cells were given media with 0.1% DMSO only. The expression of target (miR-16 and miR-125b) and housekeeping (miR-U6) genes was quantified on a Real Time PCR System (ABI 7300 quantitative RT-PCR). The data analysis indicated an upregulation tendency on miR-16 and miR-125b expression of LNCaP cells treated with finasteride. Also, the treatment induced downregulation of both microRNAs in RWPE-1 cells. MicroRNAs were not altered in PC3 cells and these results are possible due to non-functional androgen receptors in these cells. Briefly, our results suggest that finasteride modulate miR-16 and miR-125b expression in normal and tumoral prostatic cells in vitro. These results will be compared to the gene expression analysis after 10 days of finasteride treatment, towards a better understanding of the role played by finasteride in modulating microRNA expression.

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Down-regulation of micro-RNAs -208b and -499 in slow-to-fast phenotype transition and atrophy of rat skeletal muscle with heart failure.

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Heart failure (HF) is a major public health problem affecting millions of patients worldwide. As the most growing cardiovascular problem, it affects about 2 % of the western population, with the prevalence increasing sharply from 1 % in 40-year-old individuals to 10 % above the age of 75 years. Heart failure is a clinical syndrome characterized by shortness of breath and fatigue at rest or with exertion; this may in part be due to skeletal myopathy with atrophy and shift from type I "slow" to type II "fast" fibers. The identification of micro-RNAs (miRNAs) has opened up a new field of investigation to understand the molecular mechanisms which control gene expression in numerous skeletal muscle diseases. Muscle-specific miRNAs (miR-1, -133, -206, -208, -208b, and -499) have been identified and shown to be involved in a range of processes including myogenesis (proliferation, differentiation, and fiber type specification), regeneration, hypertrophy, and muscular dystrophy. Moreover, the micro-RNAs miR-208b and miR-499 are a part of a myomiR network that control MyHC expression, fiber-type and muscle performance. In this pathway, miR-208b and miR-499 are intronically encoded within slow myosin heavy-chain genes (Myh7 and Myh7b, respectively), and play redundant roles in the specification of muscle fiber identity by activating slow and repressing fast myofiber gene programs. This occurs, in part, through the miR-208b and miR-499 targeting several transcriptional repressors known to regulate muscle gene expression and function including Sox6, Pur β, Sp3 (repressors of slow muscle genes), HP-1 β, and Thrap1. The objective of the present study was to determine the expression level of the muscle-specific micro-RNAs miR-208b and miR-499 during the slow-to-fast phenotype transition and atrophy in the soleus muscle of rats with monocrotaline-induced heart failure. The most significant finding of the study was the decrease in miR-208b and miR-499 expression in the soleus muscle that was associated with an increase in Sox6 and Pur β expression. Collectively, the results suggest the intriguing possibility that decreased miR-208b and miR-499 expression contributes to the skeletal muscle alterations by de-repressing expression of genes that otherwise would control MyHC expression, fiber-type and muscle performance.

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Role of Tumor Supressive microRNAs in Pancreatic Ductal Adenocarcinoma.

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microRNAs (miRNAs) are differentially expressed in many cancers including pancreatic ductal adenocarcinoma (PDAC). We performed qPCR profiling studies of primary and mature miRNA in PDAC specimens from patients, pancreatic cancer cell lines and in the pancreas of transgenic KRASG12D transgenic mice. Major deregulation of the miRNAs was seen in the PDAC tumors with 115 of 174 expressed miRNAs (66%) increased in the tumors ($P < 0.05$). Only 5 miRNAs had a pattern of down-regulation from normal to tumor, including the pancreas specific miR-216a, miR-216b and miR-217. Results were corroborated in tissue from two different KRASG12D transgenic mouse models. Tissue specific miRNAs are down-regulated in various cancer types and reverse the malignant phenotype when miRNA mimic oligos are re-introduced into cancer cell lines. In situ hybridization of miR-216/-217 in adult, human pancreas showed that it is expressed most abundantly in acini. We hypothesize that this down-regulated cluster of miRNAs (miR-216a, miR-216b and miR-217) targets epithelial genes and might serve as a gatekeeper for the acinar-to-ductal metaplasia (ADM) seen in PDAC. A three-dimensional ADM cell culture model was employed by culturing primary mouse acini on matrigel. Ductal differentiation, (marked by increasing levels of epithelial markers, decreased levels of acinar markers and duct formation), showed decreasing levels of miR-216a, miR-216b and miR-217. Transfection of a miR-217 oligo mimic into pancreatic cell lines led to a down regulation of KRAS and CDH1 proteins. Epigenetic regulation of the cluster was studied by treating PDAC and pancreas cell lines with 5-aza 2' deoxycytidine and Trichostatin A. Current results show that the mechanism of regulation of these miRNAs does not depend on miRNA biogenesis or the epigenetic mechanisms evaluated. The ability of miR-216a, miR-216b and miR-217 to induce ADM and possibly PDAC will be studied in a miRNA knockout mouse that is currently in development.

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MicroRNAs discovery and analysis in Nile Tilapia Genome by high-throughput sequencing.

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MicroRNAs (miRNAs) are master regulators of animal development being responsible for a wide range of cellular decisions. In general, miRNAs are highly conserved in the genome of eukaryotes and considered important elements in many biological processes during development, such as cell growth, differentiation and death. Moreover, owing to the correspondence between abundance and diversity of miRNAs and increased complexity of taxonomic groups, miRNAs become key elements, both in the speciation process and as phylogenetic mark4ers for evolutionary studies. The large diversity of miRNAs identified is somewhat restricted to a few species and only a fraction of the predicted miRNAs targets has been functionally characterized. The Nile Tilapia, Oreochromis niloticus, can be considered an excellent biological model for investigating miRNAs in vertebrates due to its economic importance and genome fully sequenced. Moreover, O. niloticus belongs to the African cichlid group, which has undergone rapid and extensive adaptive radiation, being an excellent target for evolutionary studies. To date, only 26 miRNAs have been predicted by in silico analysis on O. niloticus genome but none of them was thus far validated. Therefore, our aims are to (i) identify, map and determine the genomic organization of miRNAs, and (ii) evaluate the temporal and spatial expression patterns of miRNAs and their impact on Nile Tilapia growth. Herein, solexa high throughput sequencing technology and bioinformatics analysis were used to identify miRNAs in four small RNA libraries isolated from skeletal muscle of O. niloticus. A total of 44,720,128 mapplable reads were yielded, 27,164,446 of which were related to 1667 unique miRNAs including 1318 predicted and 349 known Chordata conserved miRNAs. Nile tilapia miRNA features including length distribution and end variations were characterized. These preliminary results provide the first large scale cloning and identification of O. niloticus miRNAs. Their profiling provides the foundation for further characterization for their role in regulatory pathways of Nile tilapia, such as growth, as well as of a variety of other biological processes.

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