MicroRNAs in breast cancer – our preliminary results

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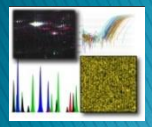
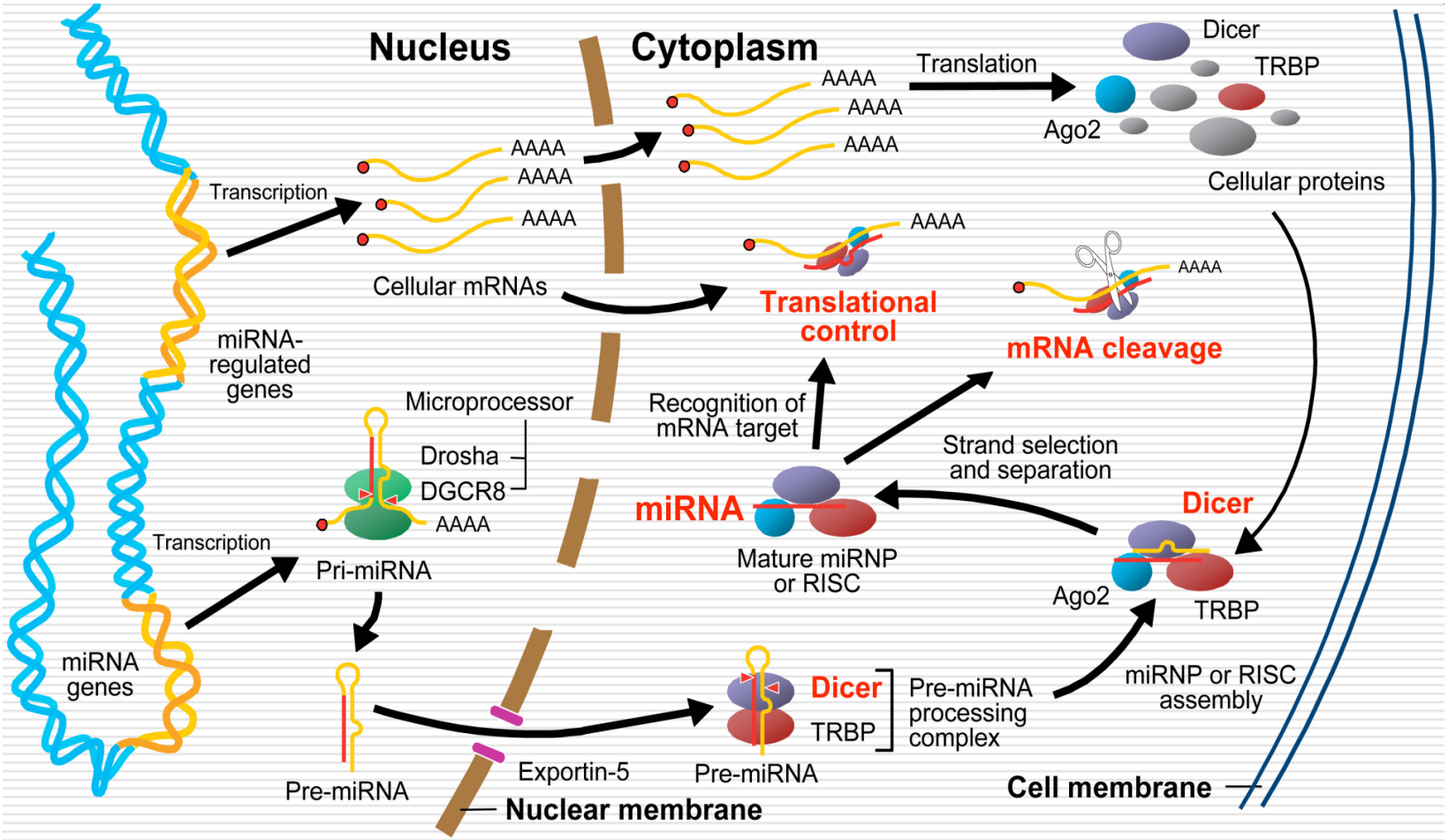
Small RNA revolution

- After the discovery of the first miRNA in the roundworm Caenorhabditis elegans, these short regulatory RNAs have been found to be an abundant class of RNAs in plants, animals, and DNA viruses;
- MicroRNAs (miRNAs) are small RNA molecules (~22 nt) that regulate gene expression post-transcriptionally;
- The Nobel Prize in Physiology or Medicine (2006) for Andrew Fire and Craig Mello for their discovery of "RNA interference – gene silencing by double-stranded RNA ".



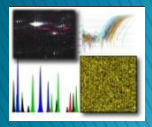
MicroRNA biogenesis



Source: Centre de recherche du Centre hospitalier universitaire de Québec CRCHUQ 2012: http://www.crchuq.ulaval.ca/en/research/researchers/4691

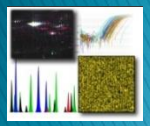
Biological role of microRNAs

- About 3% of human genes encode for miRNAs, and up to 30% of human protein coding genes may be regulated by miRNAs;
- MicroRNAs play a key role in diverse biological processes, including development, cell proliferation, differentiation, and apoptosis;
- Altered miRNA expression contribute to human disease, including cancer.



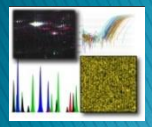
MicroRNAs and cancer

- Many miRNA genes were located at fragile sites in the genome or regions that are commonly amplified or deleted in human cancer;
- Malignant tumors and tumor cell lines were found to have widespread deregulated miRNA expression compared to normal tissues;
- In cancer, miRNAs function as regulatory molecules, acting as
  - oncogenes – miRNAs that downregulate tumor suppressors or other genes involved in cell differentiation, contributing to tumor formation by stimulating proliferation, or
  - tumor suppressors miRNAs-down-regulate different proteins with oncogenic activity.



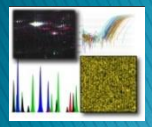
miRBase: the microRNA database

- The miRBase Sequence database contains sequences of all published mature miRNA sequences, their hairpin precursors and annotation relating to their discovery, structure and function;
- miRBase 18 contains 18226 entries representing hairpin precursor miRNAs, expressing 21643 mature miRNA products, in 168 species;
  - miRNAs are assigned sequential numerical identifiers;
  - The mature sequences are designated ‘miR’ in the database, whereas the precursor hairpins are labelled ‘mir’;
  - Distinct hairpin loci that give rise to identical mature miRNAs have numbered suffixes.



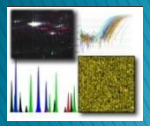
microRNA and breast cancer

- Several miRNAs are associated with breast cancer
  - miR-21, miR-155, miR-210 - up regulated
  - miR-125b, miR-145, miR-221 – down regulated
- Certain miRNA can predict the tumor subtype
  - Mattie et al., 2006
    - **ErbB2 status**: let-7f, let-7g, miR107, miR-10b, miR-154, miR195;
    - **ER/PR status**: miR-142-5p, miR200a, miR-205, miR-25
  - Lowery et al., 2009
    - **ErbB2 status**: miR-520d, miR-181c, miR-302c, miR-30e;
    - **ER+ /ER- status**: miR-342, miR-299, miR-217, miR-190, miR-218;
    - **PR + /PR- status**: miR-520g, miR-377, miR-527-518a,



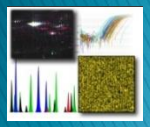
Aims of the study

- Determination and comparison of the microRNA profile in different breast cancer subtypes and normal breast tissue
- Selection of certain microRNAs that can be established as diagnostic marker for breast cancer, contributing to the improvement of breast cancer classification
- Corelation between microRNA expression profile with prognosis of the disease



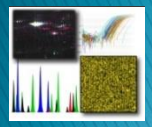
Materials and methods

- Materials
  - Normal and breast cancer tissue
- Methods
  - RNA isolation – Qiagen RNeasy kit
  - microRNA microarray (Agilent)
  - qPCR (TaqMan microRNA assays - miR-155, miR-21, miR125b, and miR-145)

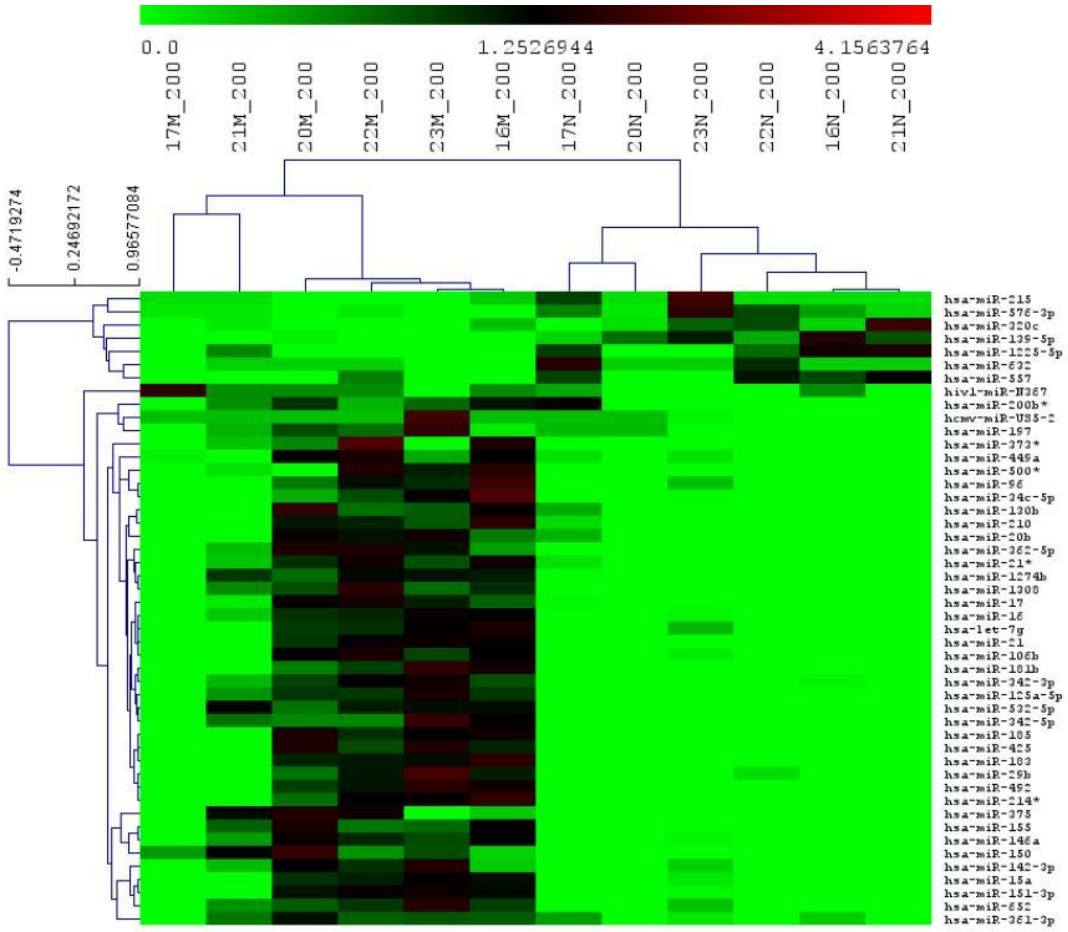


Results

- RNA was isolated from normal and cancer tissue from 39 patients
- MicroRNA microarray analysis was made for six patients
- qPCR was done for four miRNAs – miR-155, miR-21 miR-125b and miR-145



MicroRNA microarray analysis



- **Down-regulated**

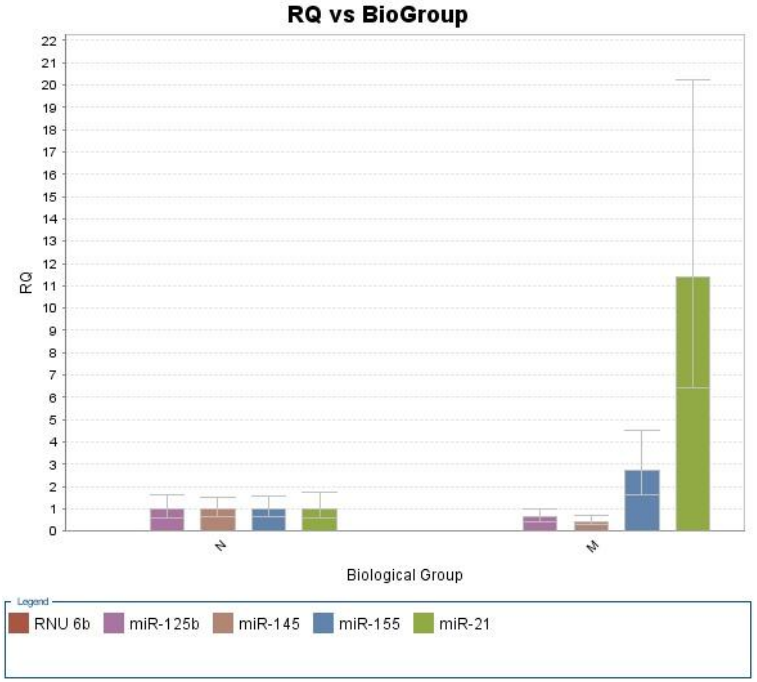
  miR-139-5p, miR-557, miR-320c, miR-632

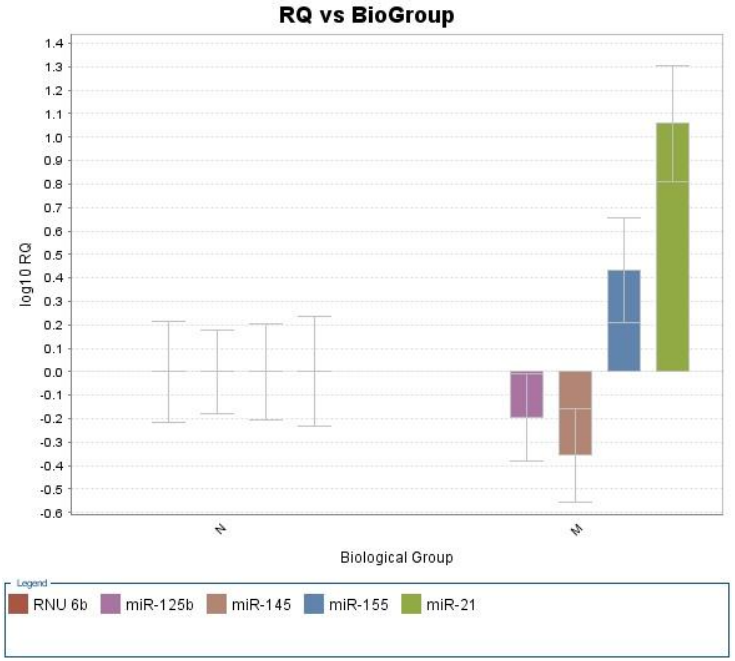
Up-regulated

miR-146a, miR-155, miR-150, miR-210, miR-425, miR-21, miR-142-3p, miR-320c, miR-342-3p, miR-342-5p

MeV – multi experimental viewer

Results – qPCR





- qPCR –ΔΔCt analysis of four microRNA

Future terms

- To include additional microRNAs as diagnostic markers for breast cancer
- Testing of particular well known deregulated microRNAs in serum of breast cancer patients and control group
- Establish the microRNA's testing as additional diagnostic and prognostic marker for breast cancer

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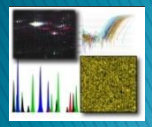

Thank you