Investigation of microRNA expression profiles related to morphological heterogeneity in triple-negative breast cancer

Koleckova M.[1], Ehrmann J.[1], Svoboda M.[3], Slaby O.[3], Bouchal J.[1], Bouchalova K.[2], Janikova M.[1], Srovnal J.[2], Radova L.[3] and Kolar Z.[1,2]

1 Department of Clinical and Molecular Pathology,
Palacky University and University Hospital Olomouc, Czech Republic
2 Institute of Molecular and Translational Medicine,
Palacky University and University Hospital Olomouc, Czech Republic
3 Central European Institute of Technology, Masaryk University Brno, Czech Republic

Palacký University
Olomouc

MUNI

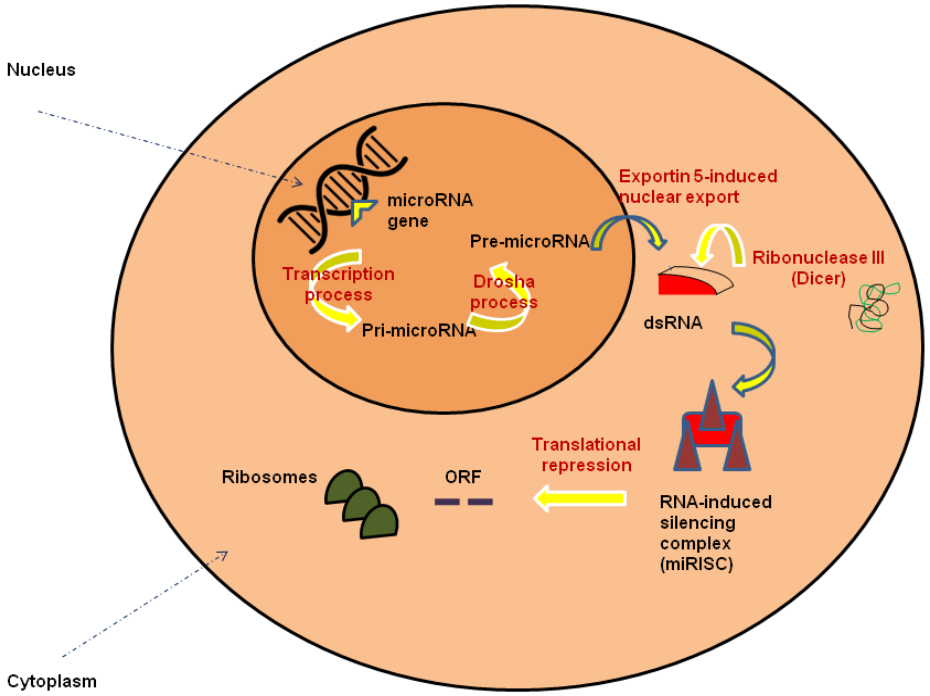
Introduction

- **Triple-negative breast cancers (TNBCs)**
  - morphologically heterogeneous group of breast cancers characterized by absent or minimal hormone receptor and HER2/neu/ERBB2 protein expression or gene amplification with specific response to therapy
- **Molecular subtypes of TNBCs (Lehmann B. D. et al., 2011)**
  - Luminal androgen receptor **(LAR)**; Basal –like 1 **(BL-1)**; Basal - like 2 **(BL-2)**; Immunomodulatory **(IM)**; Mesenchymal – like **(M)**; Mesenchymal – stem like **(MSL-L)**
- **Potential TNBC molecular targets**
  - AR, PARP, EGFR, VEGF, lncRNA, **microRNA**, siRNA, p53, CD95, PD-1/PD-L1

Introduction

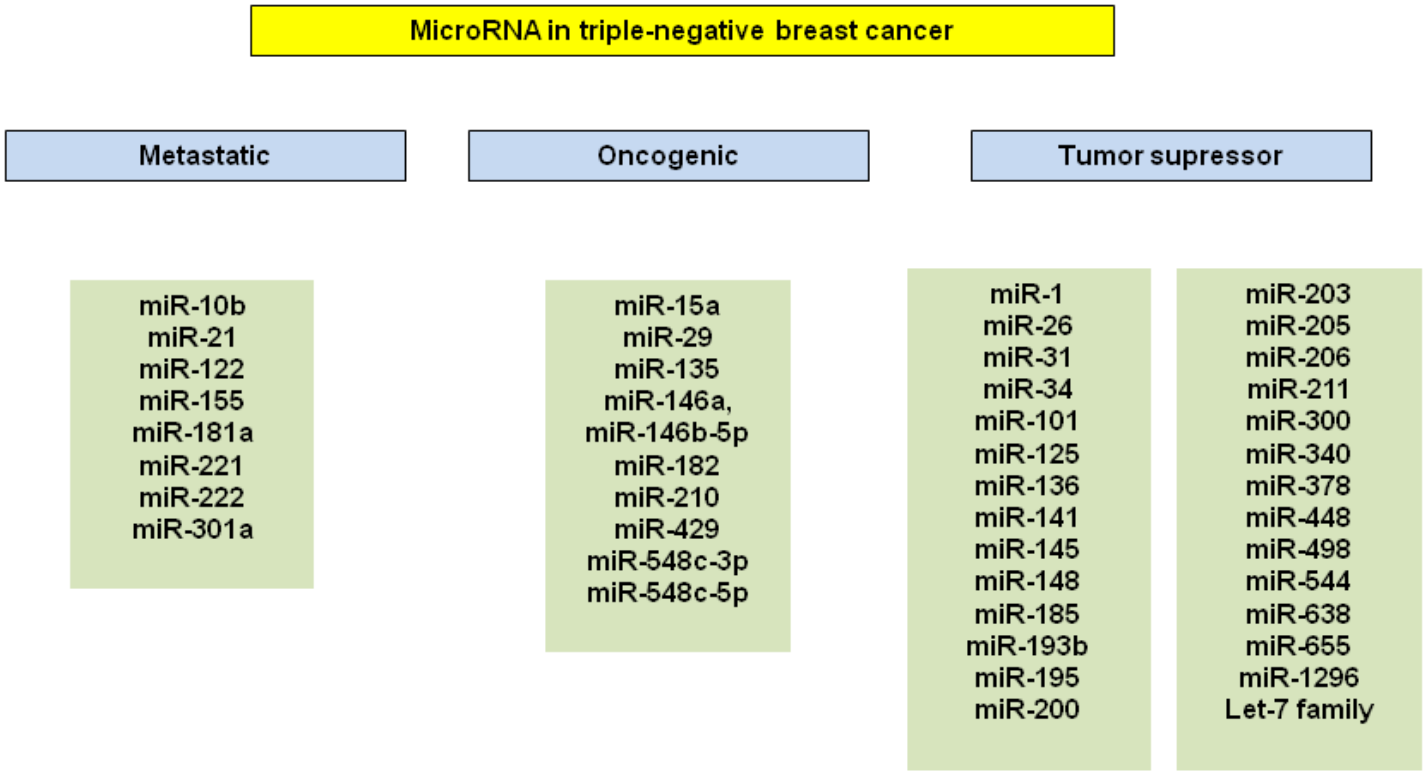
- **microRNA (miR) -** regulation of more than 50% human genes
  - non-coding RNA molecules, differing in length from 18 to 25 nucleotides
  - carcinogenesis; epithelial to mesenchymal transition (EMT); inflammation; angiogenesis; cell proliferation and migration; resistance to chemotherapy

Biosynthesis of microRNAs



Koleckova M, Janikova M, Kolar Z. MicroRNAs in triple-negative breast cancer. Neoplasma (2018); 65 (1):1-13.

Introduction



Koleckova M, Janikova M, Kolar Z. MicroRNAs in triple-negative breast cancer. Neoplasma (2018); 65 (1):1-13.

Objective and methods

- **3 953 female breast cancers** (2007-2018); **460 TNBCs** (11.6%);
- **70 TNBCs** (**absence of neoadjuvant therapy prior to surgical therapy, complete tumor volume**)
- histological tumor type (WHO classification)
- **Tumor morphology preservation:**
  - central necrosis/fibrosis
  - regions of clear cell/apocrine and/or spindle tumor cell transformation; cribriform-like pattern; small rounded neoplastic acini; monstrous tumor cells; medullary features; ductal carcinoma in situ (DCIS)
- non-neoplastic mammary gland tissue
- tumor-infiltrating lympho/plasmocytes (TILs)

Objective and methods

- 70 TNBCs FFPE tissues
  - **PALM MicroBeamlaser capture microdissection** (LCM)

    PALM RoboSoftware version 4.6 (Carl Zeiss Microscopy GmbH, Germany)
    - 10 µm breast cancer sections (6 slides); RNAse-free conditions; Cresyl Violet
    - 2 extra breast cancer sections; hematoxylin-eosin
  - **Total RNA including small RNA purification**

    AllPrep DNA/RNA FFPE kit, Qiagen, Germany
  - **Microarray analysis; Biostatistical analysis**

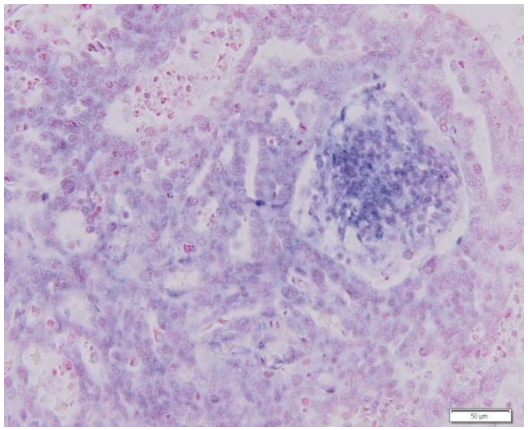
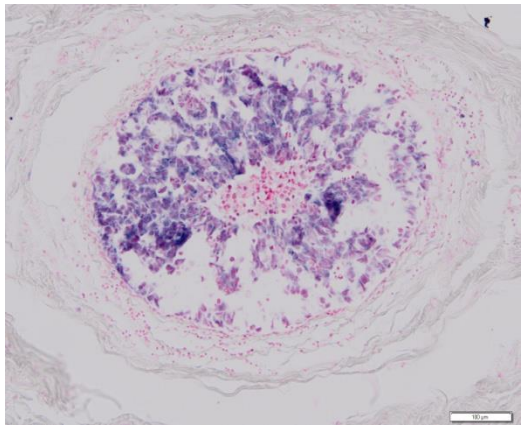
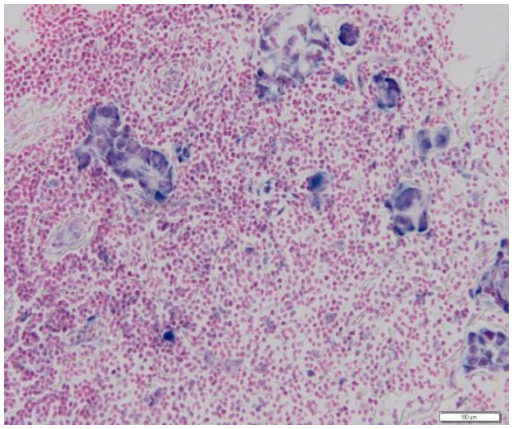
    MiRNA 4.0 Array and FlashTagTM Biotin HSR RNA Labeling Kit,

    Applied Biosystems, CA, USA ; R Core Team, ver. 3.5.0 (2018-04-23)

Objective and methods

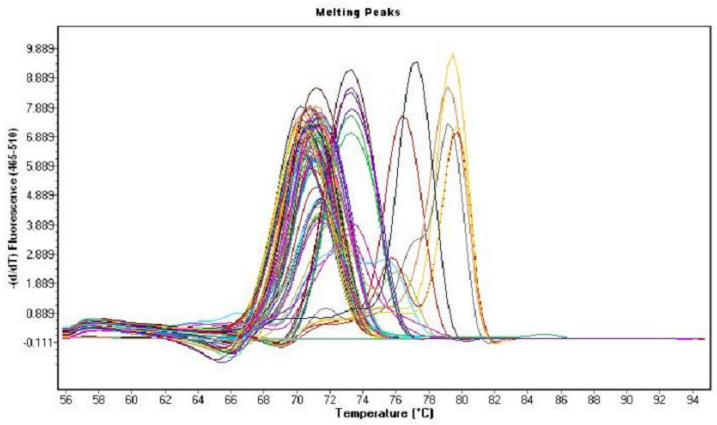
- **In-situ hybridisation**

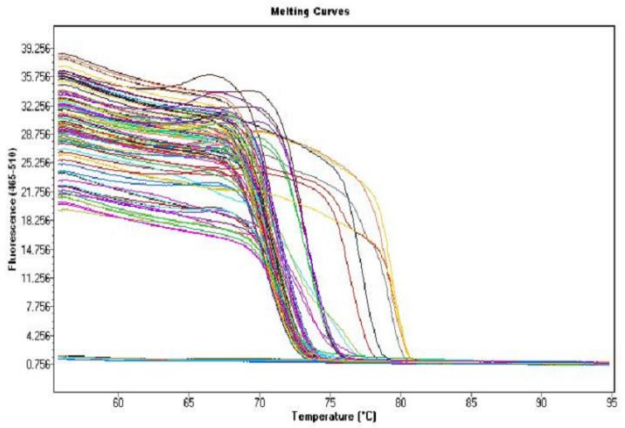
  miRCURY™ LNA™ microRNA ISH Detection Probes & Kit (Exiqon/Qiagen, Germany)

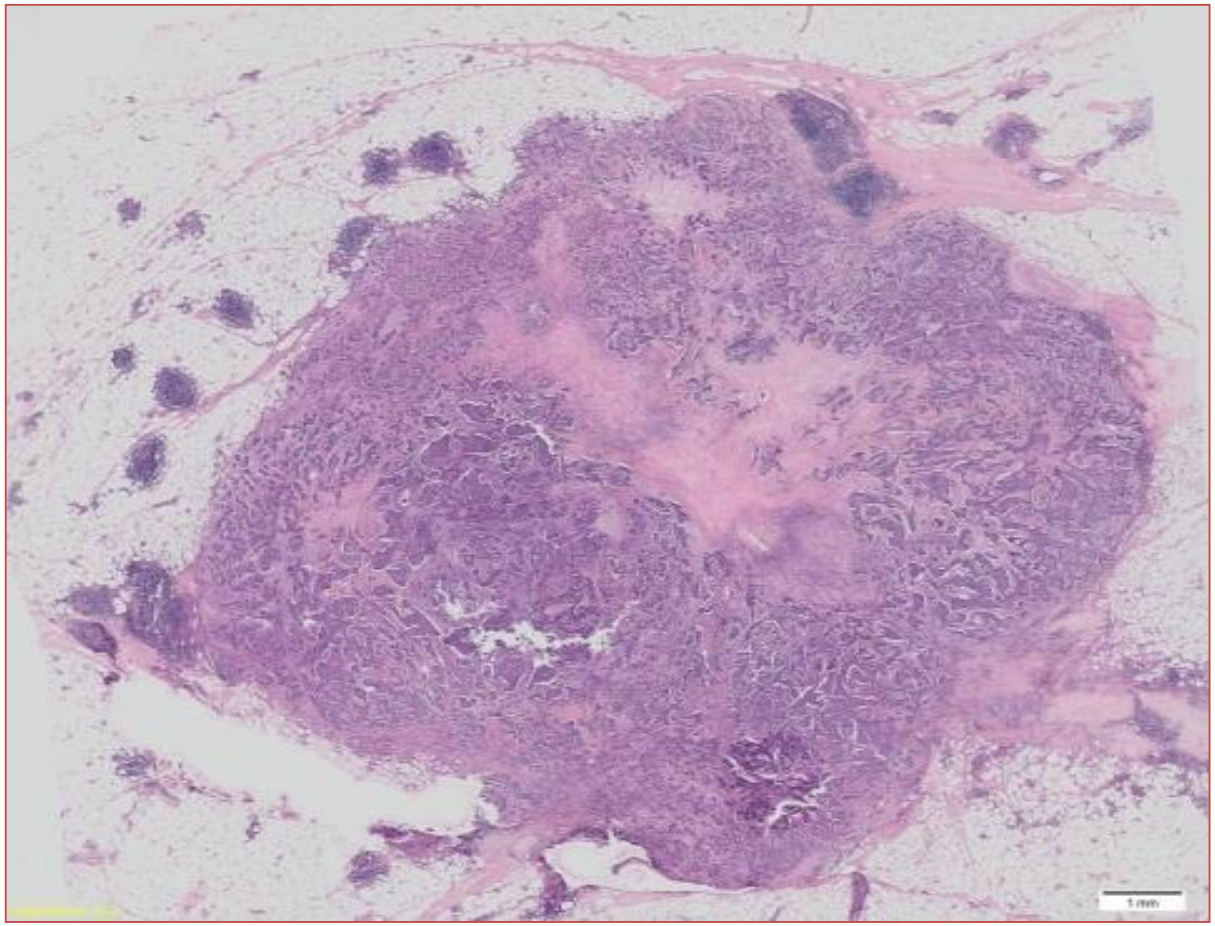
- **qRT-PCR**

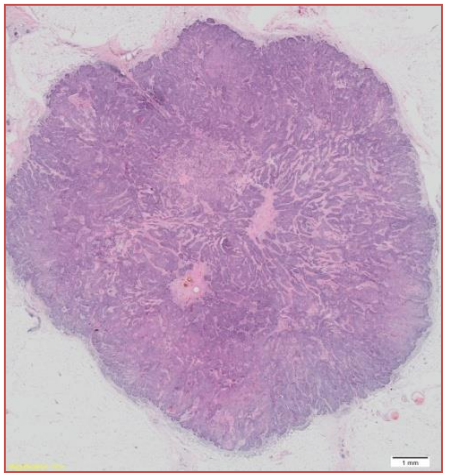
miRCURY LNA miRNA PCR System (Qiagen, Germany); Lightcycler 480 System (Roche, Switzerland)

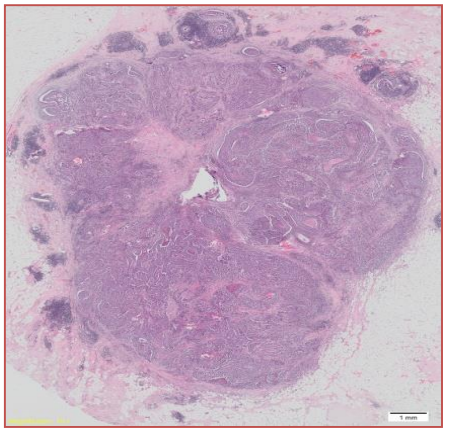




Results







Typical appearance of tumor samples (demarcation, central necrosis/fibrosis, TILs, very often morphological transformation of tumor cells from syncytial to spindle cell or clear cell pattern).

Results

- **2578 hsa-microRNAs analysed, candidate hsa-microRNAs selected**
  - corresponding with the areas of predominantly medullar, clear/apocrine and/or spindle tumor cell transformation, lymphocyte-rich, DCIS and normal mammary gland tissue morphology
  - discriminating specific morphologies

Results

- **hsa-miR-93-5p**; oncogenic and metastatic miRNA
  - discrimination of DCIS and non-neoplastic mammary gland morphology (decreased expression) from invasive tumor areas (increased expression)
- **hsa-miR-106b-5p**; oncogenic and metastatic miRNA
  - discrimination of specific tumor morphologies (increased expression) from TILs (decreased expression)
- **hsa-miR-145-5p**; tumor supressor miRNA
  - discrimination of specific tumor morphologies and TILs (decreased expression) from non-neoplastic mammary gland morphology
- **hsa-miR-200c-3p**; tumor supressor miRNA
  - discrimination of TILs

Results

- **hsa-miR-182-5p**; oncogenic miRNA
  - discrimination of specific tumor morphologies (increased expression) from DCIS and TILs (decreased expression)
- **hsa-miR-205-5p**; tumor supressor miRNA
  - discrimination of agressive tumor morphologies (spindle tumor cell; increased expression) from TILs (decreased expression) and DCIS or less agressive tumor morphologies (cribriform-like, small rounded tumor acini)
- **hsa-miR-361-5p**; tumor supressor miRNA
  - discrimination of less agressive tumor morphologies (cribriform-like, small rounded tumor acini; increased expression) from others agressive neoplastic or non-neoplastic morphologies (decreased expression)

Conclusion

- **Can miRNA expression reflect the morphological heterogeneity of TNBC?**
- **Most likely YES**
- **These morphologies have typical miRNA signature:**

Spindle tumor cell morphology: Loss: miRNA-**143**, miRNA-**205**;
Gain: miRNA- **185**, miRNA- **155**

Clear cell/apocrine tumor morphology: Loss: miRNA-**143**, miRNA-**205**;
Gain: miRNA-**182**

Medullary features: Loss: miRNA-**200**, miRNA-**143**, miRNA-205;
Gain: miRNA-**155**, miRNA-**185**

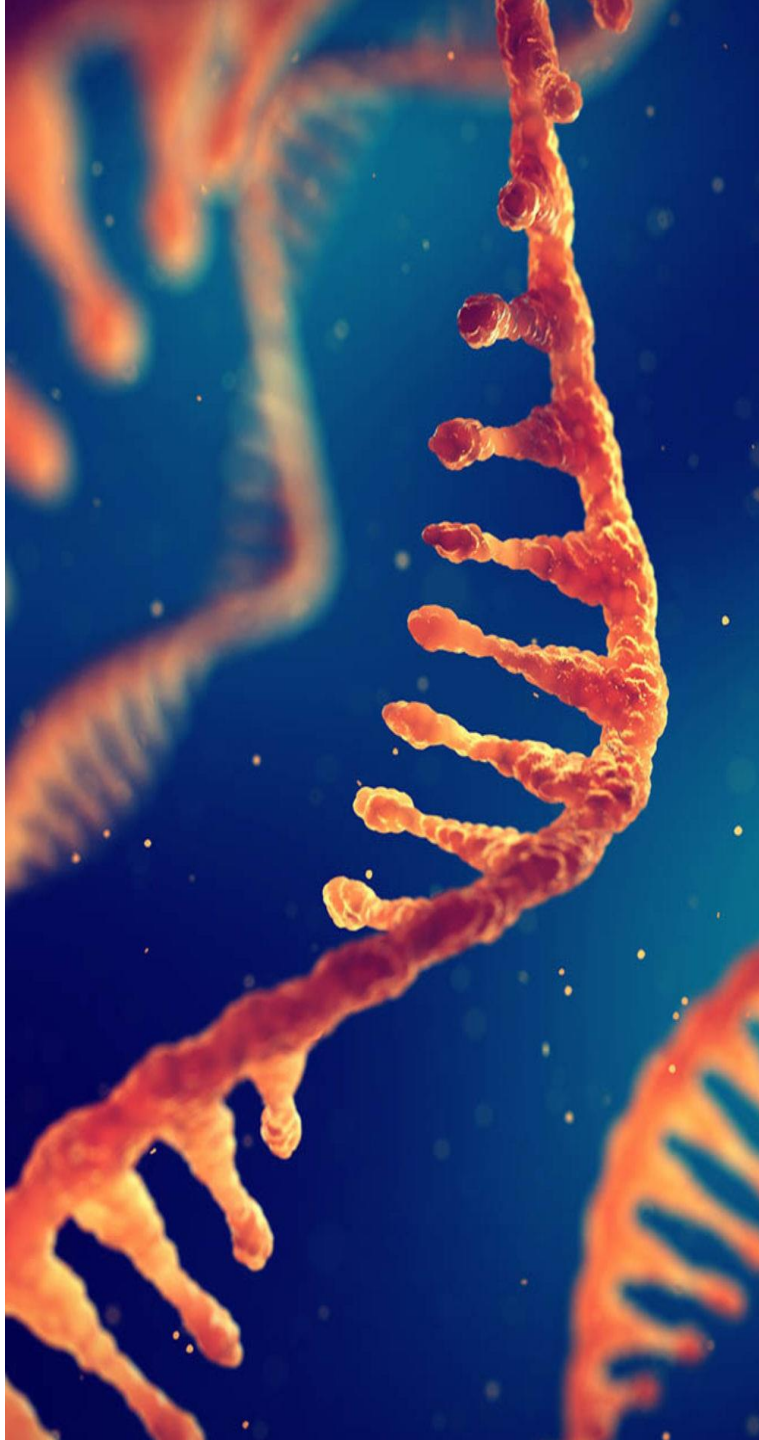
TILs: Loss: miRNA-**200**, miRNA-**143**, miRNA-**205**;
Gain: miRNA-**150**, miRNA-**155**

Lymph-node metastases: Loss: miRNA-**200**, miRNA-**150**;
Gain: miRNA-**185**

Conclusion

Keep in mind!

Changes of miRNAs can be related to proliferative or metabolic /regressive activity in tumor more than to direct up/down regulation of tumor morphology.



Thank you for your attention

Faculty of Medicine and Dentistry,
Palacky University
and University Hospital Olomouc

Central European Institute of Technology,
Masaryk University Brno

"Attitude is a little thing that makes a big difference."

W. Churchill