macrophages, although they did not influence the expression of CD80 and CD86 co-stimulatory molecules on M1 macrophages.
Conclusions: We characterized exosomes isolated from SF of patients with gonarthrosis and demonstrated that they are functionally active in their ability to stimulate the release of proinflammatory cytokines and MMPs from M1 macrophages, suggesting that they may play a role in disease progression.

BM9 MicroRNA: A Striking Player in Alzheimer's Disease
M. Akhtar[1], L. Micolucci[1], A. Giuliani[1], E. Mensà[1], M. Rippo[1], F. Olivieri[1], A. Procopio[1]
[1]Università Politecnica delle Marche, Ancona, Italy
Background: Alzheimer's disease (AD) is a common and complex age-related neurological disorder worldwide. Despite the advancements in understanding the genetic, molecular, and environmental factors of AD, there are no effective treatments to stop or reverse AD related symptoms. Early diagnosis of AD remains a big challenge as it takes a decade or more before the illness appears. Furthermore, post-mortem verification is often required. Therefore, there is an urgent need of biomarkers that can effectively detect early stage AD. MicroRNA (miRNA) is a class of non-coding RNA (~22-nt long) that regulates gene expression post-transcriptionally. Accumulating evidence suggests that miRNA plays a crucial role in neural development and differentiation. About 70% of the currently identified miRNAs are reported to be expressed in the brain. In this study, we systematically evaluated the available literature to understand the role of miRNA in AD pathogenesis.
Methods: The major biomedical databases were searched systematically to identify miRNA expression signatures in AD. Relevant articles were extracted by online searching using a combination of the following MeSH terms: microRNA or miRNA or miR and Alzheimer's Disease.
Results: We found that a number of miRNAs are deregulated in AD patients compared to the normal age-matched controls.
Conclusions: Our data suggest that miRNA signatures may have potential to be considered as a screening tool as well as a therapeutic target for AD.

BM10 Urinary Exosomes as Biomarkers in Prostate Cancer: A Pilot Study
F. Calapà[1], D. Lucchetti[1], C. Fanali[1], F. Carbone[1], A. Calarco[1], D. Pugliese[1], F. Pinto[1], V. Palmieri[1], M. De Spirito[1], P. Bassi[1], A. Sgambato[1]
[1]Università Cattolica del Sacro Cuore, Rome, Italy
Background: Exosomes circulating in biological fluids largely mirror the molecular profile of the originating cells. Given the non-invasive nature of urine collection,analysis of urinary exosome content could be useful in the identification of new biomarkers in prostate cancer. This pilot study evaluated the expression of known prostate cancer genes (PSA, PCA3 and PSMA) as potential exosomes biomarkers.
Methods: mRNA and protein contents (PSMA, CD63, CD9) of urinary exosomes collected from 20 pre-and 14 post-radical prostatectomy patients and 8 healthy age-matched men were examined. The correct isolation of exosomes was confirmed by dynamic light-scattering, transmission electron microscopy, and Western-blot analysis. Western-blot analysis, RT-PCR and nested RT-PCR were used to evaluate the expression of PSMA, PSA and PCA3 content, respectively.
Results: Expression of PSA in urinary exosomes displayed 70% sensitivity, 62.5% specificity and an overall accuracy of 67.8% in identifying prostate cancer. PCA3 sensitivity, specificity and overall accuracy were 55%, 87.5%, and 64.3%, respectively. PSMA sensitivity, specificity, and overall accuracy were 76.5%, 57.1%, and 70.8%, respectively. These values were 85%, 87.5%, and 85.7%, respectively, when all three genes were simultaneously detected in extracted exosomes. Moreover, expression of PSA, PCA3, and PSMA were no longer detectable in a significant fraction of patients after surgery (p=0.0005, p= 0.032, p=0.0002, respectively).
Conclusions: This pilot study suggests that urinary exosomes could be used as non-invasive biomarkers for prostate cancer detection, with an overall accuracy comparable to the traditional PSA. Levels of urinary exosomes are strongly related to surgery and could be adopted into post-operative surveillance.

BM11 Identification of Double-strand Genomic DNA In Colon Cancer Exosomes: Is it a Potential Non-invasive Diagnostic Tool?
F. Carbone[1], D. Lucchetti[1], P. Tomaiuolo[1], F. Calapà[1], C. Fanali[1], A. Mazzari[1], V. Palmieri[1], M. De Spirito[1], A. Crucitti[1], A. Sgambato[1]
[1]Università Cattolica del Sacro Cuore, Rome, Italy
Background: Liquid biopsy offers a promising alternative for tumor characterization and disease monitoring. Exosomes shed by cancer cells are attractive for their content stability in biological fluids and ability to mirror the molecular profile of the originating cells. This study aimed to evaluate the possibility to identify exosomes-associated DNA from colon cancer cell lines and from plasma of colorectal cancer patients (CRC pts) and to analyze their potential use in clinical settings.
Methods: Exosomes were isolated by ultracentrifugation from plasma of 10 CRC pts and from the supernatant of human colon cancer cells and were characterized. Mutation analysis of *KRAS* in DNA extracted from exosomes was evaluated by real-time PCR and confirmed by PCR-RFLP.
Results: We set up the protocols for DNA isolation from exosomes and for the analysis of *KRAS* G12V mutation by HRM analysis. *KRAS* mutation was confirmed by PCR-RFLP using *Mva*I endonuclease digestion. The analysis of exosomes DNA extracted from supernatant of colorectal cancer cells or from plasma of CRC pts confirmed that the isolated DNA displays the same mutational status of DNA extracted from cancer cell lines or from the corresponding primary tumors, respectively.
Conclusions: This pilot study suggests that DNA extracted from exosomes can be useful for the analysis of *KRAS* mutational status in colorectal cancer patients. We are currently evaluating the possibility to detect mutations in other genes (i.e., *BRAF*). Further studies are warranted to evaluate the suitability of exosome extracted DNA as a non-invasive diagnostic, predictive, and surveillance biomarker in colon cancer patients.

BM12 Asymmetric Dimethylarginine (ADMA) In Asymptomatic Cerebral Small Vessel Disease
A. Cifù[1], N. Sanvilli[1], N. Tanzi[1], C. Pistis[1], M. Fabris[1], D.E. Fontana[1], R. Giacomello[1], F. Curcio[1], G. Gigli[1], F. Janes[1]
[1]University Hospital of Udine, Udine, Italy
Background: Cerebral small vessel disease (CSVD), detected as white matter hyperintensities (WMH) on brain MRI, could be a distinct pathological entity within cerebrovascular diseases. However, the several pathogenic hypotheses so far proposed were based on very heterogeneous clinical settings. Endothelial dysfunction seems to play a pivotal role but more studies are needed to clarify it.
Methods: This is a pilot-case control study of young patients with CSVD. WMH severity was graded using the Fazekas score. Clinical history of patients and controls were negative for vascular and heart disease, classical vascular risk factors, autoimmune disorders, and coagulopathies. None of the patients reported family history of cerebrovascular disease. Blood samples were consecutively collected in the early morning and tested for inflammatory, endothelial, and prothrombotic markers.
Results: 14 patients and 12 age- and sex-matched controls were recruited (mean age 52.6±5.9 yrs). Baseline clinical characteristics did not differ between the two groups. The mean Fazekas score in patients was 2.9±0.86. Compared to controls, patients did not display higher levels of common inflammatory markers (C-reactive protein, fibrinogen, IL-6, IL10) and plasma prothrombotic factors (PAI, vWF, tPA, PAF-AH, homocysteine). In contrast, the levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide, were significantly increased in patients compared to controls (122.2±33.7 µmol/l versus 93.4±32.8 µmol/l; p=0.04).

Conclusions: Our pilot study suggests that ADMA, a known marker of endothelial dysfunction, may represent a new biomarker to identify asymptomatic CSVD, indicating the pathway of nitric oxide may be involved in endothelial dysfunction in this clinical setting.

BM13 Platelet Activation Affects Pre-mRNA Maturation of a Group of Transcripts Useful as Markers of Acute Coronary Syndromes

R. Tarallo[1], G. Nassa[1], G. Giurato[1], G. Cimmino[2], G. Bruno[1], F. Rizzo[1], L. Ricciardi[1], A. Salvati[1], G. Marchese[3], P. Golino[2], A. Weisz[1]
[1]University of Salerno, Baronissi, Italy; [2]Second University of Naples, Naples, Italy; [3]Genomix4Life Srl, University of Salerno, Baronissi, Italy

Background: Platelets represent a major player in the process of intravascular thrombus formation. Despite significant advancements in antithrombotic therapy, current strategies still fail to prevent thrombotic coronary events in a substantial number of patients, indicating that the complex mechanisms modulating platelet response during activation are not fully elucidated. The evidence that platelets are capable of *de novo* protein synthesis has raised the issue of whether and how these resident mRNAs are regulated in circulating platelets. Among the various mechanisms potentially involved, mRNA splicing may be potentially relevant.
Methods: Purified platelet-rich plasma from healthy volunteers were collected and *in vitro* activated with collagen or thrombin receptor activating peptide. Transcriptome analysis by RNA-Seq and *in silico* intron retention analysis were applied to search for splicing events affected by platelet activation. HiRIEF LC-MS allowed platelet proteome characterization at deep coverage to investigate a possible correlation between splicing events and protein levels.
Results: Extensive computational analysis following RNA-Seq revealed several splicing events occurring in activated platelets. By applying unbiased proteogenomics, we correlated intron retention events in quiescent platelets to exon-exon junctions frequency after activation. In this way we identified a set of transcripts presenting reduced intron retention and high peptide representation at exon-exon junctions in activated vs resting platelets.
Conclusions: The observed results indicate that pre-mRNA maturation of platelet-specific transcripts could be monitored and used as marker of platelet activation in acute coronary syndromes.
Acknowledgement: Research supported by MIUR (FIRB RBFR12W5V5_003), MOH (GR-2011-02347781), CNR (Flagship Project InterOmics) and Genomix4Life.

BM14 *Lactobacillus reuteri*: Production and Characterization of Membrane Vesicles for Future Health Applications

R. Grande[1], C. Celia[1], A. Stringaro[2], L. Di Marzio[1], M. Di Marcantonio[1], M. Colone[2], M. Locatelli[1], L. Savino[1], V. Puca[3], L. Hall-Stoodley[4], P. Stoodley[4], R. Muraro[1], G. Mincione[1]
[1]University "G.d'Annunzio" Chieti-Pescara, Chieti, Italy; [2]Istituto Superiore di Sanità, Rome, Italy; [3]University of L'Aquila, L'Aquila, Italy; [4]The Ohio State University, Columbus, Ohio, USA

Background: The probiotic *Lactobacillus reuteri* is effective against infantile colic, alleviation of eczema and *Helicobacter pylori* colonization. *L.reuteri* develops biofilm *in vitro*, producing factors that give health benefit to the host although the mechanism by which commensal bacteria communicate with the host remains unclear. The purpose of this study was to detect and characterize membrane vesicles (MVs), bilayer structures containing several molecules generated by bacteria, produced by *L.reuteri* DSM 17938.
Methods: The structure of MVs was evaluated by transmission and scanning electron microscope analysis. MVs were subsequently isolated by biofilm (bMVs) and planktonic (pMVs) phenotypes by ultracentrifugation and physicochemically characterized by dynamic light scattering (DLS) analysis. An enzymatic treatment was performed to determine MVs composition. eDNA was detected and quantified using the Quant-iTTMPicoGreendsDNA assay and NanoDropUV-VIS spectrophotometer. Proteins associated with MVs were extracted and quantified by BCA assay.
Results: PicoGreen showed that eDNA was associated with MVs and that its concentration is higher in bMVs than in pMVs, although an inverse correlation was found in the protein concentration, suggesting a different role of MVs in the two phenotypes. The enzymatic treatment showed that lipids and proteins represent the main structural components of MVs. The DLS analysis demonstrated that *L.reuteri* generates MVs with sizes in the nanometer range and a broad size distribution.
Conclusions: *L.reuteri* produces MVs whose main components are lipids and proteins; eDNA is also associated to MVs. The biological activity and composition of MVs may represent the starting point for future applications in the development of vesicles-based therapeutic systems.

BM15 Methylation Analysis of Protocadherin Genes in Pancreatic Adenocarcinoma

M. Curia[1], F. Fantini[1], F. Tavano[2], F. Di Mola[3], R. Lattanzio[1], M. Di Marcantonio[1], L. Savino[1], M. Piantelli[1], P. Battista[1], P. Di Sebastiano[3], A. Cama[1]
[1]University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy; [2]Division of Gastroenterology and Research Laboratory, San Giovanni Rotondo, Italy; [3]Division of Surgical Oncology "SS Annunziata" Hospital, Chieti, Italy

Background: Pancreatic cancer is one of the most lethal malignancies and somatic mutations of protocadherins have been identified. Some of them were recently identified as tumor-suppressor genes; in particular, *PCDH10* is a functional tumor suppressor gene frequently silenced by methylation in multiple carcinomas. Our goal is to evaluate the prognostic significance of protocadherin methylation in pancreatic adenocarcinoma so we studied methylation in primary pancreatic cancers. Functional assays with a *PCDH10* re-expressing pancreatic cancer cell line is in progress to characterize its biological effects in pancreatic tumorigenicity.
Methods: 38 ductal pancreatic adenocarcinomas were recruited in "Casa sollievo della sofferenza" Hospital, S.Giovanni Rotondo. DNA was extracted from tumor tissue and treated with bisulfite solution. COBRA analysis and bisulfite genomic sequencing were used to determine the presence of methylated CpG in the promoter regions. A functional assay using AspC-1 and Capan-2 pancreatic cancer cell lines transfected with full length PCDH10 will assess the effect of *PCDH10* on pancreatic cancer cell growth.
Results: Cases were studied for *PCDHAC2*, *PCDHGC5* and *PCDH10* methylation status and immunohistochemistry (IHC). Results showed no *PCDHAC2* methylation pattern and ubiquitous methylation of *PCDHGC5* in all cases analysed. *PCDH10* analysis showed a partial methylation pattern in 9/18 and no methylation in 9/18 cases analysed. IHC analysis detected PCDHAC2 expression in 6% of cases analysed and PCDHGC5 in 30%. IHC of PCDH10 is in progress.
Conclusions: The identification of aberrantly hypermethylated and silenced genes will have diagnostic, prognostic, and therapeutic applications. *PCDH10* may be a potential target gene for cancer therapy.

BM16 Primary Pleuro-Pulmunary Synovial Sarcoma: A Single-Center 13-Year Experience

S. Di Russo[1], G. De Luca[2], C. Moscatello[1], M. Di Marcantonio[1], G. Mincione[1], A. Marchetti[3], P. Battista[1], F. Mucilli[4]
[1]University of Chieti-Pescara, Chieti, Italy; [2]Istitute of pathology, "S.S. Annunziata" Hospital, Chieti, Italy; [3]University-Foundation, CeSI Biotech, Center of Predictive Molecular medicine, Chieti, Italy; [4]"S.S. Annunziata" Hospital, Chieti, Italy

Background: Primary pleural and pulmonary synovial sarcomas (PPSSs) are very rare and aggressive neoplasms that affect adults. The oncologic characteristics, treatment, and prognosis for PPSSs are not well defined because of a paucity of data. Dysregulation of Wnt/β-catenin and EGFR pathways leads to tumorigenesis with poor prognosis. We investigated the involvement of Wnt/ β-catenin and EGFR signaling in PPSSs.