# PRESENTATIONS

## PROTEOMIC CHANGES INDUCED BY LOW-INTENSITY ENDURANCE EXERCISE IN MDX MOUSE QUADRICEPS: CORRELATION WITH REDUCTION OF MUSCLE DEGENERATION

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Previous study showed that low-intensity endurance exercise induced a significant recovery of damaged skeletal muscle in mdx mice, probably by reducing the degeneration of dystrophic muscle[1]. In order to explore the molecular basis of this observation, we perfomed a proteomic analysis to evaluate changes in proteins profiling of quadriceps dystrophic muscles of exercised versus sedentary *mdx* mice. Four protein spots were found significantly changed and were identified as three isoforms of Carbonic anhydrase 3 (CA3) and as superoxide dismutase [Cu-Zn] (SODC). Protein levels of CA3 isoforms were found significantly up-regulated in quadriceps of sedentary *mdx* mice (MDX-Sed) and were completely restored to wild type values in quadriceps of exercised *mdx* mice (MDX-Ex). Protein levels of SODC were found down-regulated in quadriceps of sedentary *mdx* mice and were significantly restored to wild type values in quadriceps of exercised *mdx* mice. These proteomic data, validated by Western blot analysis, indicate that low-intensity endurance exercise, by modulating some proteins involved in oxidative stress defense, may in part contribute to reduce the reported cell degeneration in *mdx* muscles[1].
Further investigations are needed to better define the extension of proteins change in MDX-Ex versus MDX-Sed mice and its correlation with the recovery of damaged fibers in MDX-Ex mice.

1. Frinchi M *et al.* Int J Sports Med 2014, 35:19-27.

## ALTERED CYTOSKELETAL ORGANIZATION MODULATES THE PHENOTYPIC VARIABILITY IN A MURINE MODEL OF OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta (OI) is a heritable bone disease characterized by a wide spectrum of clinical outcomes ranging from very mild to lethal. Identical molecular defects are associated in OI with phenotypic variability, a recurrent feature in hereditary diseases. Brtl$^{+/-}$ mice, a model of dominant OI, show either a moderately severe or a lethal outcome associated with the same Gly349Cys substitution present in the 1 chain of type I collagen, thus they are a valid tool to investigate the molecular basis of OI phenotypic variability. Our previous data revealed that the intracellular machinery in lethal mice is less effective at coping with the intracellular retention of mutant collagen favoring up-regulation of molecules involved in apoptosis with respect to the mice with a moderately severe outcome in which chaperone up-regulation is predominant[1,2]. Here we demonstrated by immunohistochemistry with fluorescent phalloidin, a specific marker for actin filaments, the presence of an abnormal cytoskeleton in calvarial bone, long bone, skin and lung in Brtl$^{+/-}$ mice with lethal outcome (ML). In the same tissues in the ML mice we detected also a reduced number of integrin-based focal adhesions (FAs). In long bone of ML mice collagen deposition was impaired as well as TGF- signalling and ML calvarial osteoblasts revealed reduce cell proliferation as well as decrease expression of the early osteogenic marker Runx2. Thus in ML animals alterated actin filaments and FAs negatively affects cell differentiation, extracellular matrix composition, cell signaling and cell–matrix interaction. The consequence of this dysregulation have an impact on the bone structural properties: ML bones showed significantly reduced lenght, BV/TV and cortical thickness with respect to wild type both by histomorphometric and nanoCT analysis. Importantly, abnormal cytoskeletal assembly was detected in fibroblasts obtained from lethal, but not from non-lethal, OI patients carrying a substitution at the same glycine[3].
Our study identify cytoskeleton as a phenotypic modulator and as a potential novel target for OI treatment.
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1.Forlino A et al. Proteomics, 2007, 7:1877-1891.
2. Bianchi L et al. J. Proteomics, 2012, 75:4717-4733.
3. Bianchi L et al. Human Molecular Genetics, 2015 in press.

## *IN VITRO* EFFECTS OF OF AgNPs EXOPOLYSACCHARIDE FROM *KLEBSIELLA OXYTOCA* DSM29614 ON BREAST CANCER CELLS

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Bacterial exopolysaccharides (EPSs), high-molecular-weight sugar polymers surrounding bacterial cells , have achieved considerable attention because of their potential applications in many fields, including biomedicine, exspecially as antineoplastic molecules. A *Klebsiella oxytoca* DSM 29614 (KO) strain, ex BAS-10, produces an EPS made of rhamnose, glucuronic acid and galactose, which shows metal-binding properties[1,2]. More recently, it has been reported that KO in the presence of $AgNO_3$ is able to synthesize Ag nanoparticles (AgNPs) embedded in branched EPS (AgNPs-EPS). The AgNPs-EPS, produced under aerobic and anaerobic conditions, contain $Ag^{+1}$ and $Ag^0$ that could have different biological activity[3]. The present study was aimed to assess the cytotoxic effects of AgNPs-EPS, produced under aerobic and anaerobic conditions, on breast cancer cell line SK-BR3. The responses to the AgNPs-EPS treatments revealed a dose dependent behavior resulting at 5 g/ml in a inhibition of cell proliferation rate of 50% (IC50), dramatic

morphological changes consistent with apoptotic features and extensive proteomic modulation . The most important effects were obtained by aerobically biosynthesized AgNPs-EPS treatment, due to the major release of $Ag^{+1}$, as verified by voltammetry analysis. Proteomic analysis showed modulation of several proteins related to oxidative stress and apoptotic and mitochondrial pathways. Taken together, these results provide new important elements in support of the potential antitumoral activity of AgNPs-EPS.

1. Leone S et al. Eur J Org Chem 2007, 31:5183-5189.
2. Baldi F et al. N Biotechnol 2011, 29:74-78.
3. Battistel D et al. Talanta. 2015, 132:294-300.

## COMPARATIVE PROFILING BY PROTEOMICS AND ZYMOGRAPHIC ACTIVITIES OF TUMORAL AND NON-TUMORAL CELL LINES

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The extracellular matrix (ECM) underlying epithelial tissues is involved in the maintenance of cell polarity and homeostasis. ECM is a dynamic structure under the regulated remodeling of its components. The major enzymes responsible of matrix degradation are the matrix metalloproteinases (MMPs), a well known family of zinc-dependent endopeptidases. Much attention has been focused on MMP-2 and MMP-9 because of their ability to degrade type IV collagen, a major constituent of basement membranes.
A deregulated proteolysis of ECM molecules may cause the alteration of cell polarity and may contribute to the disruption of cell–cell and cell–ECM adhesions, promoting cancer progression. These alterations are responsible for a poor prognosis, and a positive correlation between the increase of MMPs and the degree of malignancy has also been observed FOR many tumor histotypes. To approach these issues on in vitro models, we performed a comparative study, between a couple of tumoral and non-tumoral mammary cell lines and a couple of thyroid cell lines derived respectively from a benign and malignant cancer.
This experimental approach, based on scanning electron microscopy, on proteomic analysis and on gelatin zimography, highlighted a similar profiling of the two differential couples of cell lines: that is between malignant and non-malignant cells respectively, regardless of their histological origin.
In particular, it was observed that the cell lines derived from aggressive cancers, when compared with their non-malignant counterpart, showed an increased secretion of MMPs, a cell shape highly pleomorphic and a higher expression of protein clusters potentially associated with invasion and metastasis. The analysis of the interactions between the expression of MMPs and of selected proteomic clusters have offered important indication on the complex network existing between neoplastic cells and their environment.
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## IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN ATHEROSCLEROTIC PATIENTS WITH TYPE 2 DIABETES

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Atherosclerosis is a form of chronic inflammation characterized by the accumulation of lipids and fibrous elements in medium and large arteries that represents a major cause of death and disability in people with diabetes.
The aim of this study is to identify differentially expressed plasma proteins between patients with or without type 2 diabetes undergoing carotid endarterectomy, by applying two-dimensional electrophoresis analysis coupled with mass spectrometry.
Briefly, 14 plasma samples from diabetic patients and 15 plasma samples from non-diabetic patients were subjected to a low-abundance proteins enrichment step using hexapeptide combinatorial ligand libraries (ProteoMiner™ enrichment kit, Bio-Rad Laboratories) followed by two-dimensional electrophoresis. This analytical technique allows resolving hundreds of different protein isoforms according to both isoelectric point and molecular weight. Protein profiles were compared by using PD-Quest software (Bio-Rad Laboratories) and spots of interest were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). Differential analysis was validated by 1D- and 2D-western blotting. An interaction map was made using String 10 (http://string-db.org/).
A panel of proteins differentially expressed between the two groups of atherosclerotic patients have been identified. Among them, there are fibrinogen beta and gamma chains, complement c1r, c3 and c4-B subcomponents, alpha-1-antitrypsin, vitronectin and some apolipoproteins. Preliminary results on predicted protein-protein interactions suggest that vitronectin could play a role in modulating fibrinolysis, complement dependent immune responses and other pathways in diabetes. Actually, identification of markers in diabetic patients could be of interest for clarifying the biochemical mechanisms underlying the strong association between diabetes and atherosclerosis.
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## EXTRACELLULAR VESICLES SHED BY A375 MELANOMA CELLS, CONTAIN H1° RNA AND RNA-BINDING PROTEINS

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Extracellular vesicles (EVs) are shed in the extracellular environment by both prokaryotes and eukaryotes. Although pro-