european journal of histochemistry

a journal of functional cytology

ISSN 1121-760X volume 59/supplement 2 2015

PROCEEDINGS OF THE XXXV NATIONAL MEETING OF THE ITALIAN SOCIETY FOR THE STUDY OF CONNECTIVE TISSUES (SISC)

Palermo, October 15-17, 2015 University of Palermo - Palazzo Steri

President

Antonio Rossi

Local Organizing Committee

Ida Pucci Minafra, Patrizia Cancemi, Gianluca Di Cara, Rosa Musso, Anna Filosto, Nadia N. Albanese

Scientific Committee

Antonio Rossi, Mario Raspanti, Maria Enrica Tira, Patrizia Cancemi, Salvatore Campo, Federica Boraldi, Marilena Formato, Marco Franchi, Michele Spin



under the auspices of the University of Pavia, Italy



Published by PAGEPress, Pavia, Italy

Editorial Office:

via G. Belli 7, 27100 Pavia, Italy

Phone: +39.0382.1751762 - Fax: +39.0382.1750481

E-mail: info@pagepress.org

Printed quarterly by:

Press Up s.r.l.

via La Spezia, 118/C 00055 - Ladispoli (RM)

Phone and Fax: +39.076.15.27.351

Annual Subscriptions

Europe: Euro 200

All other Countries: \$ 250

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy.

Cancellations must be received before the end of September to take effect at the end of the same year.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means (electronic, electrostatic, magnetic type, mechanical, photocopying or otherwise) without written permission by the Publishers.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Recognized by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.



Disclaimer. Whilst every effort is made by the publishers and the editorial board to see that no inaccurate or misleading data, opinion or statement appears in this journal, they wish to make it clear that the data and opinions appearing in the articles or advertisements herein are the responsibility of the contributor or advisor concerned. Accordingly, the publisher, the editorial board and their respective employees, officers and agents accept no liability whatsoever for the consequences of any inaccurate or misleading data, opinion or statement.

european journal of histochemistry

a journal of functional cytology

ISSN 1121-760X volume 59/supplement 2 2015

Editor

C. Pellicciari Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani" Università di Pavia



under the auspices of the University of Pavia, Italy



European Journal of Histochemistry a journal of functional cytology

The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published until 1979 under the title of Rivista di Istochimica Normale e Patologica, from 1980 to 1990 as Basic and Applied Histochemistry and in 1991 as European Journal of Basic and Applied Histochemistry. It is published under the auspices of the University of Pavia, Italy.

The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Book Reviews, Views and Comments, concerning investigations performed with the aid of biophysical, biochemical, molecular-biological, enzymatic, immunohistochemical, cytometric, and image analysis techniques.

Areas of particular interest to the *European Journal* of *Histochemistry* include:

- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

Editor in Chief

Carlo Pellicciari (University of Pavia, Italy)

Editor

Marco Biggiogera (University of Pavia, Italy)

Managing Editors

C.A. Redi (University of Pavia, Italy) E. Solcia (University of Pavia, Italy)

for Japan: M. Fukuda (Fukui Medical School, Fukui, Japan)

for Latin America: R.F. Donoso (University of Chile) for USA and Canada: A. Nanci (University of Montreal, Canada)

Assistant Editor

Manuela Malatesta (University of Verona, Italy)

Managing Board of the Italian Society of Histochemistry for the years 2014-2017

Carlo Pellicciari (President), University of Pavia, Italy

Roberto Di Primio (vice-President), University of Ancona, Italy

Paolo Onori (Member), University of Rome "La Sapienza", Italy

Paola Sirigu (Member), University of Cagliari, Italy Silvia Modina (Secretary), University of Milan, Italy Fiorenza De Bernardi (past-President), University of Milan, Italy

Editorial Board

B. Bilinska, Warsaw; E. Bonucci, Rome; G. Bottiroli, Pavia; L. Cocco, Bologna; A. Diaspro, Genoa; E. Falcieri, Urbino; P. Hozak, Prague; Z. Kmiec, Gdansk; A. Lauria, Milan; H.G. Lee, Cleveland; F.A. Manzoli, Bologna; N.M. Maraldi, Bologna; F.J. Medina, Madrid; G. Meola, Milan; S. Modina, Milan; S. Muller, Strasbourg; P. Panula, Helsinki; M. Pavelka, Vienna; G. Perry, San Antonio; G. Rindi, Rome; A. Riva, Cagliari; C. Sotelo, Paris; P.G. Suh, South Korea; J.P. Tremblay, Quebec.

Members appointed by Scientific Societies

E. Bàcsy (Histochemical Section of the Society of the Hungarian Anatomists), B. Bloch (Societé Française de Microscopie Electronique), A. Lòpez Bravo (Federacion Iberoamericana de Biologia Celular y Molecular), B. Bilinska (Polish Histochemical and Cytochemical Society), M.A. Nahir (Israel Society for Histochemistry and Cytochemistry), D. Onicescu (Romanian Society of Histochemistry and Cytochemistry), W. Ovtscharoff (Histochemical Section of the Society of Anatomy, Histology and Embryology of Bulgaria), P. Panula (Finnish Society of Histochemistry and Cytochemistry), L. J. Pelliniemi (Finnish Society of Histochemistry and Cytochemistry), J. Renau Pigueras (Spanish Society for Cell Biology), B. Rodé (Histochemical and Cytochemical Section of Croatian Society of Anatomists), M. Rosety (Sociedad Iberoamericana de Histoguimica y Citoquimica).

2014 Impact factor: 2.042. [©]JCR Thomson Reuters

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

R. Musso¹, G. Di Cara¹, N.N. Albanese¹, P. Cancemi^{1,2}, D. Martini³, C. Giordano^{1,4}, I. Pucci Minafra¹

²Centro di OncoBiologia Sperimentale, University of Palermo, La Maddalena Cancer Center, Palermo, Italy; ²Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy; ³Department of Human Anatomical Sciences and Physiopathology of Locomotor Apparatus, Human Anatomy Section, University of Bologna, Bologna, Italy; ⁴Section of Cardio-Respiratory and Endocrine-Metabolic Diseases, Biomedical Department of Internal and Specialist Medicine (Di.Bi.M.I.S.), University of Palermo, Palermo, Italy

E-mail: rosi82.m@libero.it

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.

The work was co-funded by the Italian 5x1000 to COBS.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN ATHEROSCLEROTIC PATIENTS WITH TYPE 2 DIABETES

G. Nieddu¹, A.J. Lepedda¹, O. Lobina¹, S. Rocchiccioli², N. Ucciferri², M. Idini¹, T.H.Q. Nguyen¹, P. De Muro¹, R. Spirito³, A. Guarino³, M. Formato¹

¹Dipartimento di Scienze Biomediche, University of Sassari, Sassari, Italy, ²Istituto di Fisiologia Clinica, National Research Council, Italy and ³Centro Cardiologico "F. Monzino", IRCCS, Milano, Italy.

E-mail: ganieddu@uniss.it

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 lowabundance 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 clr, 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

This work was supported by Regione Autonoma della Sardegna (Legge Regionale 7 Agosto 2007, N.7 – Bando 2010 - Grant no. CRP-26789 and P.O.R. Sardegna F.S.E. 2007/2013, Asse IV Capitale Umano - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività I.3.1) and by Fondazione Banco di Sardegna.

association between diabetes and atherosclerosis.

EXTRACELLULAR VESICLES SHED BY A375 MELANOMA CELLS, CONTAIN H1° RNA AND RNA-BINDING PROTEINS

<u>G. Schiera</u>¹, C.M. Di Liegro¹, O. Colletta^{1,2}, V. Puleo^{1,2}, G. Di Cara³, P. Cancemi¹, A. Fricano^{1,2}, I. Di Liegro²

¹Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche; ²Dipartimento di Biomedicina sperimentale e Neuroscienze cliniche, University of Palermo, ³Centro di Oncobiologia Sperimentale (COBS), Palermo, Italy

E-mail: gabriella.schiera@unipa.it

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