

CURRICULUM VITAE

MONICA GIANNOTTA

Work address

FIRC Institute of Molecular Oncology Foundation (IFOM)
Vascular biology Laboratory
Via Adamello, 16
20139 Milan
Italy

Place/ date of birth: Basel, Switzerland; 4 July, 1977
Citizenship: Italian
Marital status: Married

Languages: Italian, Mother tongue,
English, good spoken and written

Professional qualifications

- 2010 – present **Post doc** at FIRC Institute of Molecular Oncology Foundation
IFOM
Title: Targeting endothelial junctions as a novel strategy to
increase stem cell engraftment in dystrophic muscles
Supervisors: Prof. Elisabetta Dejana
- 2005 – 2009 **PhD**, Open University, UK (Life Sci) and University College of
London.
Supervisor: Prof. Mark Marsh, Laboratory for Molecular Cell Biology
Unit of Genomic Approaches to Membrane Traffic, Laboratory of
Membrane Traffic, DCBO
Title: A new signalling pathway leads the KDEL receptor to activate
Src on the Golgi membranes
Supervisors: Dr. Michele Sallese and Dr. Alberto Luini
- 2001 – 2002 BSc thesis (at Pharmacia, Milan)
Title: Purification and characterisation of a chimeric HIV reverse
transcriptase enzyme: an engineered drug target substitute for
telomerase.
Part of a patent (Pharmacia) (WO/2003/095605): A chimeric reverse
transcriptase and methods for identifying telomerase inhibitors.
Internal Supervisor: Prof. Santi Spampinato (Bologna University)
External Supervisors: M. Maria Flocco (Pharmacia), Arndt
Schnuchel (Pharmacia)

1997 – 2003 **BSc** in Biotechnology, Bologna University (final mark: 110/110 cum laude)

Employment

- Jan 2012 - present:** Co-Co-Pro contract, FIRC Institute of Molecular Oncology. Foundation (IFOM), Milan, Italy
- Feb 2010 - Jan 2012:** Fellowship, FIRC Institute of Molecular Oncology. Foundation (IFOM), Milan, Italy
- Jan 2008 - Jan 2010:** Fellowship, Unit of Genomic Approaches to Membrane Traffic, Department of Cell Biology and Oncology (DCBO), Consorzio Mario Negri Sud (CMNS), Santa Maria Imbaro (Chieti), Italy.
- Jan 2005 - Dec 2007:** Fellowship, Fondazione Italiana per la Ricerca sul Cancro (FIRC), in the Unit of Genomic Approaches to Membrane Traffic, DCBO, CMNS.
- Feb 2004 - Dec 2004:** Predoctoral Fellowship, Unit of Genomic Approaches to Membrane Traffic, DCBO, CMNS.
- Nov 2003 - Jan 2004:** Fellowship, Department of Experimental Oncology and Surgery, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, Milan, Italy.

Publications

Cancino J, Capalbo A, Di Campli A, **Giannotta M**, Rizzo R, Jung JE, Di Martino R, Persico M, Heinklein P, Sallese M, Luini A. Control Systems of Membrane Transport at the Interface between the Endoplasmic Reticulum and the Golgi. *DEVELOPMENTAL CELL*. 2014 Aug 11;30(3):280-94.

Giannotta M, Benedetti S, Tedesco FS, Corada M, Trani M, D'Antuono R, Millet Q, Orsenigo F, Gálvez BG, Cossu G, **Dejana E**. (2014) Targeting endothelial junctional adhesion molecule-A/ EPAC/ Rap-1 axis as a novel strategy to increase stem cell engraftment in dystrophic muscles. *EMBO MOLECULAR MEDICINE*. 2014 Feb;6(2):239-58.

Ruggiero C, Cancino J, **Giannotta M**, Sallese M. (2014) Signaling initiated by the secretory compartment. *METHODS IN ENZYMOLOGY* 534, 133-54 doi: 10.1016/B978-0-12-397926-1.00008-1.

Giannotta M, Trani M, Dejana E (2013) VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *DEVELOPMENTAL CELL* 26, 441-54

Xu H, Oliveira-Sales EB, McBride F, Liu B, Hewinson J, Toward M, Hendy EB, Graham D, Dominiczak AF, **Giannotta M**, Waki H, Ascione R, Paton JFR, Kasparov S

(2012). Upregulation of junctional adhesion molecule-A is a putative prognostic marker of hypertension. *CARDIOVASCULAR RESEARCH*, ISSN: 0008-6363

Giannotta M, Ruggiero C, Grossi M, Cancino J, Capitani M, Pulvirenti T, Consoli GML, Geraci C, Fanelli F, Luini A, and Sallese M (2012). The KDEL receptor couplesto Gaq/11 to activate Src kinases and regulate transport through the Golgi. *EMBOJOURNAL*, vol. 31, p. 2869-2881, ISSN: 0261-4189, doi:10.1038/emboj.2012.134

Murakami M, Giampietro C, **Giannotta M**, Corada M, Torselli I, Orsenigo F, Cocito A, d'Ario G, Mazzarol G, Confalonieri S, Di Fiore PP, Dejana E (2011). Abrogation of Junctional Adhesion Molecule-A Expression Induces Cell Apoptosis and Reduces Breast Cancer Progression. *PLOS ONE*, vol. 6, ISSN:1932-6203, doi:10.1371/journal.pone.0021242

Sallese M, **Giannotta M**, Luini A (2009). Coordination of the secretory compartments via inter-organelle signalling. *SEMINARS IN CELL & DEVELOPMENTAL BIOLOGY*, vol. 20, p. 801-809, ISSN: 1084-9521, doi:10.1016/j.semcdb.2009.04.004

Pulvirenti T*, **Giannotta M***, Capestrano M, Capitani M, Pisanu A, Polishchuk R, SanPietro E, Beznoussenko GV, Mironov AA, Turacchio G, Hsu VW, Sallese M and Luini A (2008). A traffic-activated Golgi-based signalling circuit coordinates the secretory pathway. *NATURE CELL BIOLOGY*, vol. 10, p. 912-922, ISSN: 1465-7392, doi:10.1038/ncb1751 * These authors contributed equally to this work.

Computer Skills

Windows Vista, Microsoft Office, Explorer and Microsoft Outlook
Vector NTI programme for sequence alignment and vector construction

Awards

Fondazione Italiana per la Ricerca sul Cancro (FIRC) Research Fellowship
(Jan 2005 - Dec 2007).

Premio Nazionale Salento 2008, awarded by the Mayor of Sogliano Cavour (LE), Italy

Research experience

PhD thesis: **A new signalling pathway leads the KDEL receptor to activate Src on the Golgi membranes**

During my PhD thesis, I have carried out basic research on a signalling pathway initiated by a Golgi-localised signal–receptor system that senses, and is activated by, the arrival of transport carriers at the Golgi complex from the endoplasmic reticulum (ER). This triggering signal is provided by the ER chaperones: as they reach and enter the Golgi within ER-to-Golgi carriers, they bind to and ‘activate’ the KDEL receptor through their KDEL motif. The KDEL receptor is a seven-transmembrane-domain-containing protein that once activated, dimerises, changes conformation within the membrane, and recruits the coatamer protein (COP)I trafficking machinery. The chaperones are then recycled to the ER with the KDEL receptor. The KDEL receptor binding of these chaperones in the Golgi activates the Src family kinases (SFKs), and hence a SFK-dependent tyrosine phosphorylation cascade on the Golgi complex. In turn, this activates the intra-Golgi transport machinery, balancing the membrane input to the Golgi that initiates the whole process with increased membrane output from the Golgi. We published the initial description of this Golgi-localised signal–receptor system in late 2008 (*Nat Cell Biol*, **10**, 912-22), and the follow-up manuscript from the rest of my PhD project has been published on *EMBO Journal*.

BSc thesis: **Purification and characterisation of a chimeric HIV reverse transcriptase enzyme: an engineered drug target substitute for telomerase.**

For my BSc thesis project, I developed an expression system and a purification scheme that allowed large amounts of pure chimeric protein to be obtained for a screening assay and for structural characterisation. I expressed the chimeric protein at high levels in *Escherichia coli* as inclusion bodies, and after solubilisation I purified the protein with immobilised metal chelation affinity chromatography and gel filtration chromatography. After protein refolding, I obtained an active enzyme, which I characterised using biochemical and biophysical techniques, including isothermal titration calorimetry, analytical centrifugation, and liquid chromatography/ mass spectrometry. I then assessed the enzymatic activity of the pure protein using an ELISA-RT assay. In addition, I applied molecular biology techniques, such as PCR-based mutagenesis, to mutate all of the cysteines in the protein to serines, to avoid aggregation, degradation and concentration problems. Based on the results obtained during my thesis project, the chimeric HIV RT protein became available for identification of specific inhibitors and for structural studies.

Laboratory techniques

- Cell culture, with human lymphocytes, fibroblasts, haematopoietic stem cells, and HeLa, HEK-293T, COS-7 A375 and immortalised or primary endothelial cells
- Animal handling
- Transient and stable transfection of plasmids, siRNAs using lipid reagents and microporation and shRNA by lentivirus infection
- Transmigration, adhesion and permeability assay
- Protein co-immunoprecipitation
- Nucleic acid extraction, purification and electrophoresis analysis
- PCR
- Cloning techniques using plasmid vectors for protein expression
- Small, media and large scale cell culture (petri, to flask, to fermenter)
- Recombinant protein expression and extraction from bacteria
- Protein purification by chromatography (gel filtration, ion exchange) and tag binding (His) using AKTA explorer (Amersham Pharmacia)
- Spectrophotometric methods to determine protein and DNA concentrations
- SDS-PAGE
- Western blotting
- Protein/protein and protein/ligand interaction studies by isothermal titration calorimetry (ITC)
- Protein biophysical studies by analytical centrifugation (AUC)
- Enzymatic assays (ELISA) to test activities
- Immunohistochemistry and immunofluorescence techniques (widefield and confocal microscopy, time lapse)

Congresses and Workshops attended

Gordon Research Conference "Cell Contact & Adhesion", 2013, Lucca, Italy

OPTISTEM/ENDOSTEM Annual Meeting, 2013. Ischia, Naples, Italy

Invited speaker: Targeting endothelial Junctional Adhesion Molecule-A as a novel strategy to increase stem-cell engraftment in dystrophic muscles

Frontiers in cardiac and vascular regeneration, 2012, Trieste, Italy

EMBO Conference, Cellular signaling & Molecular Medicine, 2012, Cavtat-Dubrovnik, Croatia

OPTISTEM/ENDOSTEM Annual Meeting, 2012. Sitges, Barcellona. Spain

Invited speaker: Increasing stem cell homing by manipulating endothelial cell-to-cell junctions

OPTISTEM/ENDOSTEM Annual Meeting, 2011. Fontevraud. France

The European Summer School on Stem Cells & Regenerative Medicine VI, 2010, Hydra. Greece

Poster presentation: Increasing homing of mouse muscle mesoangioblasts

First Joint Annual Consortium Meeting, 2010. Stresa. Italy

Poster presentation: Increasing homing of mouse muscle mesoangioblasts

Golgi Meeting 2008: Membrane trafficking in global cellular responses. Pavia. Italy.

Poster presentation: Involvement of a heterotrimeric G protein in traffic-dependent KDEL-receptor signalling.

1st Joint PhD Student Conference (CMNS/ UCL, London), 2007. Guardigliare, Italy.

Speaker: Novel KDEL-receptor interactors in traffic-dependent KDEL- receptor signalling.

Keystone Symposia: Cell Signalling and Proteomics, 2007. Steamboat Springs, Colorado.

Poster presentation: Novel KDEL-receptor interactors in traffic-dependent KDEL-receptor signalling.

7th Young Scientists Meeting, German Society for Cell Biology: Vesicular Trafficking, 2005. Jena, Germany.

Poster presentation: A traffic-activated Golgi-based signalling circuit co-ordinates the secretory pathway.

FEBS Advanced Course, 2005: Lipid-protein interactions in signalling and membrane trafficking. S. Maria Imbaro, Italy.

Poster presentation: A traffic-activated Golgi-based signalling circuit co-ordinates the secretory pathway

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