PhD Students' works

6th Edition

Progress Report 2011

Doctorate in Genetics, Oncology and Clinical Medicine

GenOMeC

University of Siena

Santa Chiara Scuola Superiore

PhD Students' works

6th Edition

Progress Report 2011

Doctorate in Genetics, Oncology and Clinical Medicine

GenOMeC

Biotechnology Department
and
Department of Clinical Medicine and Immunological Sciences
Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry
Department of Human Pathology and Oncology
Department of Surgery
and
A.O.U.S. Azienda Ospedaliera Universitaria Senese
Human Health Foudation Onlus, Spoleto
I.T.T. Istituto Toscano Tumori
S.H.R.O. Sbarro Health Research Organization, USA
Siena Biotech
Sorta srl

Layout: Ufficio Comunicazione Online
Print: Centro Stampa di Ateneo

This initiative is aimed to spread the information on the research activities of PhD students in our academic community.

The pamphlet is in English in order to promote Doctoral Schools of our University at international level, with particular attention to those foreign institutions with which we have signed international cooperation agreements. Moreover, it could also be useful to foster new agreements with foreign partners.

The Rector
Prof. Angelo Riccaboni


SIGILLVM · SENTRUM · SUNIVERSITATIS

This pamphlet was created to regroup and present together the research activities of the students of the Doctorate in Genetics, Oncology and Clinical Medicine (GenOMeC) in order to spread information about the work of the students and to promote the collaboration on research projects.

The first pages illustrate the activity of the “annual progress report day”. This event takes place at the end of each academic year and is dedicated to the presentation of both the research projects proposed by the new entered students and the annual progress reports of the older students. The pamphlet continues with the presentation of the research abstracts of the **57 PhD students**. Finally, the last pages are dedicated to the “thesis discussion days” and qualification of “Doctor Europaeus”.

I wish to dedicate this pamphlet to the PhD students who represent the “mainstay” of the Institution that we call “University” with their continuous daily work, their perseverance and motivation.

The director of the School
Prof. Alessandra Renieri


SENTRUM·SUNIVERSI

The Doctorate in Genetics, Oncology and Clinical Medicine (GenOMeC) derives from the fusion between the Doctoral School in Oncology and Genetics and the Doctoral School in Biomedicine and Immunological Sciences of the University of Siena. This new Doctorate was ranked first in a regional selection called “Progetto Pegaso” aimed to create in Tuscany a Doctorate of international level by integrating research and educational centers of excellence. We successfully created this network involving the three Tuscan Universities (Siena, Florence and Pisa), a company (Siena Biotech), a spin-off of the University of Pisa (SORTA srl), Siena University Hospital (AOUS) and other research Institutes (Istituto Toscano Tumori, ITT). This link with industries will promote translational medicine and will facilitate professional employment of students after PhD.

The Doctorate is aimed to educate students on the molecular basis and clinical, diagnostic, and therapeutic aspects of monogenic and multifactorial diseases (resulting from the interaction between genes and environment), including cancer.
The Doctorate is divided into eight educational trainings:
1) **Medical Genetics**, focused on the genetics and physiopathology of intellectual disabilities, hereditary tumours and rare syndromes;
2) **Genetics and physiopathology of skeletal disorders**, focused on the genetics and physiopathology of osteopathies;
3) **Genetics and physiopathology of atherosclerosis**, focused on the genetics and physiopathology of cardiovascular and atherothrombosis-related diseases;
4) **Pharmacogenetics and clinical pharmacology**, focused on the knowledge of how drugs vary with patient’s genome and aimed to develop “individual” therapies;
5) **Surgical oncology**, focused on clinical-epidemiological-observational studies of thoracic and intestinal tumors;
6) **Hematological and dermatological oncology**, focused on clinical-epidemiological-observational studies of melanoma and blood tumors;
7) **Oncological Genetics, focused on the identification of new targets and diagnostic**, prognostic and predictive markers for oncological therapies;
8) **Clinical Immunology**, focused on the molecular basis and clinical aspects of rare immunological disorders such as idiopathic pulmonary fibrosis, alpha 1-antitrypsin deficiency and rare autoimmune rheumatic diseases.

The **Faculty Board** (Collegio) is composed by:
- **15 teachers** from the University of **Siena**: Francesca Ariani, Francesco Cetta, Serenella Civitelli, Michele Fimiani, Mauro Galeazzi, Antonio Giordano, Theodora Hadjistilianou, Franco Laghi Pasini, Monica Bocchia, Francesca Mari, Clelia Daniela Anna Miracco, Ranuccio Nuti, Alessandra Renieri, Paola Rottoli, Piero Tanganelli;
- **3 teachers** from the University of **Florence**: Laura Papi (Associate Professor MED/03-Medical Genetics), Roberta Sestini (Associate Professor MED/03-Medical Genetics), Mirca Marini (Ricercatore M-EDF/02-Methods and Education of Sport Activities);
- **3 teachers** from the University of **Pisa**: Lucia Migliore Papi (Full Professor MED/03-Medical Genetics), Antonella Cecchettini (Ricercatore, BIO/13-Applied Biology), Alessandro Corti (Ricercatore MED/04-General Pathology);

The following additional teachers compose the **Council of the School** (Consiglio):
- **8 teachers** from the University of **Siena**: Capecchi Leopoldo, Giordano Nicola, Lauria Francesco, Marzocca Giuseppe, Puccetti Luca, Roviello Franco, Rubegni Pietro, Toti Paolo;
- **3 teachers** from the University of **Florence**: Maurizio Genuardi (Full Professor MED/03-

Medical Genetics), Sabrina Giglio (Associate Professor MED/03-Medical Genetics), Gabriella Vannelli (Full Professor BIO/16-Human Anatomy);
- **3 teachers** from the University of **Pisa**: Alfonso Pompella (Full Professor MED/04-General Pathology), Salvetti Alessandra (Associate Professor BIO13-Applied Biology), De Tata Vincenzo (Ricercatore, MED/04-General Pathology);
- **2 teachers** from Istituto Toscano Tumori **ITT**: Lucio Luzzatto e Mario Chiarello;
- **1 teacher** from **Siena Biotech**: Giovanni Gaviraghi;
- **1 teacher** from **AOUS**: Michele Maio.

The above reported composition confers to the School a **unique characterization crossing the 3 Tuscany Universities** focused on Pathology, Applied Biology and Medical Genetics.

On the basis of research activity the Doctorate has signed **8 International Cooperation Agreements** with the following Universities:
Bilkent University, Ankara, Turkey;
Duisburg-Essen University, Germany;
Freiburg University, Germany;
Greenwood Genetic Center, Greenwood, South Carolina, USA;
Kentucky University, Lexington, USA;
Radboud University of Nijmegen, The Netherlands;
St. Kliment Ochridski University, Sofia, Bulgaria.
University of Lausanne, Switzerland

The Doctorate in Genetics, Oncology and Clinical Medicine at the University of Siena trains students to carry out research in Medical Genetics, Clinical and Molecular Oncology and Clinical Medicine over a **three years** program. The aim of this Doctorate is to train researchers who will be able to plan and develop competitive research proposals. The Doctorate has a dedicated **web site** at the following address: http://www.unisi.it/ricerca/dottorationweb/genetica_medica/.

In this site it is possible to find general information on the Doctorate, seminar activities, research projects, and PhD students scientific “identity cards”.
The Doctorate, on the basis of the high quality of the education activities and the internationalization of the scientific and teaching courses, has been selected by a external board as one of the Doctorates of the University of Siena belonging to the **Graduate College Santa Chiara**. The Doctorates of the Graduate College join in multidisciplinary and international research projects, creating a centre of high qualification for postgraduate education. The PhD students of the Graduate College are called “santachiarini” and are provided with the additional title of the Graduate College and the stay in the University residences. Residences of the Graduate College are situated in the old town. In these buildings teaching activities, conferences and interdisciplinary courses and seminars take place, but the most innovative aspect is that they are informal places for meetings where PhD students and teachers can stay and eat together.

The following tables refer to Doctoral School **selection and attractiveness**, efficiency and **resources**, level of **internationalization**, PhD students and PhD **productivity**, and **employability**. These tables refer to Oncology and Genetics Doctoral School only. Updated tables of the whole Doctorate will be published on the website.The raw data they refer to the tables can be found at Anagrafe della Ricerca Università di Siena, and at the “PhD students” section of the web site of the School:
http://www.unisi.it/ricerca/dottorationweb/genetica_medica/students.htm

DOCTORAL SCHOOL SELECTION AND ATTRACTIVNESS

CYCLE	DEGREE	APPLICATIONS	TOTAL APPLICATIONS	AVAILABLE POSITIONS
XXIV	SIENA	9	69	12
	OUTSIDE SIENA	52		
	OUTSIDE ITALY	8		
XXV	SIENA	8	39	6
	OUTSIDE SIENA	26		
	OUTSIDE ITALY	5		
XXVI	SIENA	9	41	6
	OUTSIDE SIENA	31		
	OUTSIDE ITALY	1		
XXVII	SIENA	Inscription shifted to February 1, 2012		
	OUTSIDE SIENA			
	OUTSIDE ITALY			

DOCTORAL SCHOOL EXTERNAL RESOURCES

Cycle	ITT	MIUR	Regione Toscana "Progetto Pegaso"	University Department and AOUS	Total
XXIV	1	1	0	0	2
XXV	0	1	0	0	1
XXVI	0	1	0	0	1
XXVII	0	1	3	4	8

DOCTORAL SCHOOL EFFICIENCY AND RESOURCES

Year	n° fellowship	n° PhD	Ratio
2008	3	3	1
2009	3	5	1,67
2010	3	12	4
2011	8	16	2

DOCORAL SCHOOL EMPLOYABILITY AND LEVEL OF INTERNATIONALIZATION

CYCLE	N° of PhD		PhD employed
	TOTAL	DOCTOR EUROPEAUS	
XX	4	0	4
XXI	8	3	6
XXII	9	3	7
XXIII	7	0	5

DOCTORAL SCHOOL PRODUCTIVITY (PhD students)

Cycle	PhD students	Publications total number	Publications average number (n°/ PhD n°)	Total IF	Average IF (IF/ PhD n°)	Abstracts of conference total number	Abstracts of conference average number
XXIV	Bruccheri MG	25	2.5	162.323	16.23	105	10.5
	Colecchia D						
	Conti D						
	Crucianelli F						
	Disciglio V						
	Forte IM						
	Mucciolo M						
	Lorenzi B						
	Pacifici M						
	Zangari R						
XXV	Cozzi M.	7	1.167	23.456	.91	61	10.167
	Fontani A.						
	Grillo E.						
	Guercio V.						
	Livide G.						
	Olabinjo O.						
XXVI	Casini N.	16	2.67	54.84	9.14	23	3.83
	Fallerini C						
	Granato F.						
	Martellucci J						
	Mencarelli MA						
	Rizzo V						

DOCTORAL SCHOOL PRODUCTIVITY (PhD)

Cycle	PhD students	Publications total number	Publications average number (n°/PhD n°)	Total IF	Average IF (IF/ PhD n°)	Abstracts of conference total number	Abstracts of conference average number
XX	Barellini L	13	3.25	48.99	12.25	19	4.75
	Causarano V						
	Roberti A						
	Sampieri K						
XXI	Artuso R	49	7	149.935	21.42	38	5.4
	Chessa A						
	Katzaki E						
	Malagnino G						
	Mancino M						
	Squillaro T						
	Vignoli M						
XXII	Abbadessa G	39	4.87	163.2	20.4	48	6
	Benoni S						
	Guarnaccia V						
	Khadang B						
	Marcocci E						
	Papa F						
	Rosseto A						
	Rizzolio F						
XXIII	Amenduni M.	29	3,625	119.816	14.977	81	10,125
	Azzarà A.						
	De Filippis R.						
	La Montagna R.						
	Laviano P.						
	Mischitelli M.						
	Parri V.						
	Rondinella D.						

Index

Annual Progress Report
Oncology and Genetics Doctoral School
Biomedicine and Immunological Sciences Doctoral School
September 13, 2011 Centro Didattico S Maria alle Scotte, room 15

8.45 Welcome Addresses
Angelo Riccaboni, Rector of the University of Siena
Alessandra Renieri, Director of Oncology and Genetics Doctoral School
Franco Laghi Pasini, Director of Biomedicine and Immunological Sciences Doctoral School

9.00 Progress report of the 3rd year, XXIV cycle (10 minutes for each one)
Chairmen: Mario Chiariello and Francesca Ariani

Bruccheri Maria Grazia (A. Renieri) postponed at 15.00 (videoconferenze from Catania)
A reciprocal translocation t(2;8)(q31;q24) and a de novo 2p22.3p21 interstitial deletion in a patient with developmental delay, jont laxity, peculiar facial features

Colecchia David (M. Chiariello)
Involvement of the Erk8 in autophagy

Conti Daniele (A. Giordano)
Small molecules mimicking the spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents

Crucianelli Francesca (S. Civitelli - M. Genuardi)
Analysis of constitutional epigenetic changes in colorectal cancer and in multiple primary tumors (MPT) by MS-MLPA technique

Disciglio Vittoria (F. Mari - A Renieri)
The mirror effect of deletion and duplication on 16p11.2

Forte Iris Maria (A. Giordano)
Pharmacological targeting of p53 effectively indices apoptosis in malignant mesothelioma cell lines

Landi Claudia (P. Rottoli)
Proteomic analysis of interstitial lung diseases

Lorenzi Bruno (F. Cetta)
New insights in the physiology of the internal anal sphincter in patients treated with neoadjuvant therapy for rectal cancer: effects of chemoradiotherapy, role of the MRI and properties of the interstitial cells of Cajal

Mucciolo Mafalda (F. Mari - A Renieri)
Copy number variations and modifier genes in Rett syndrome

Pacifici Marco (A. Giordano) postponed at 15.20 (videoconferenze from New Orleans)
MicroRNA signature of HIV-associated neurological disorders

Zangari Rosalia (F. Cetta)
Mannose-binding lectin (MBL): the complement pathway activation in the pathogenesis of brain injury

11.00 Progress report of the 2nd year, XXV cycle (10 minutes for each one)
Chairmen: Serenella Civitelli and Francesca Mari

Cozzi Martina (A. Giordano)
New small molecule inhibitors of SRC as potential candidates for cancer therapy

Fontani Andrea (G. Tanzini)
Outcome of surgical treatment of colorectal cancer in the elderly

Grillo Elisa (F. Ariani - A. Renieri)
Rett Database Network: an integrated clinical and genetic network of Rett syndrome databases

Guercio Valentina (F. Cetta)
Incidence of cancer in cantenarians and in elderly with Alzheimer's disease

Livide Gabriella (F. Ariani - A. Renieri)
Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA

Olabinjo Olayinka (A. Giordano) postponed at 15.40 (videoconference from Philadelphia)
Bioinformatics approach: data analysis on tumor suppressor genes in cancer initiation and progression

12.30 Progress report of the 1st year, XXVI cycle (5 minutes for each one)
Chairmen: Paola Rottoli and Francesco Cetta

Casini Nadia (A. Giordano)
Role of the Cdk9/Cyc complex during muscular differentiation in normal and pathological conditions

Fallerini Chiara (A. Renieri)
Advances in Alport syndrome diagnosis using next generation sequencing

Granato Felice (F. Cetta) postponed at 16.00 (videoconference from Glasgow)
Thymomas have an increased risk of developing additional malignancies: lack of immunologic surveillance?

Martellucci Jacopo (F. Cetta)
Accuracy of transrectal ultrasound after preoperative radiochemotherapy compared to computed tomography and magnetic resonance in locally advanced rectal cancer

Mencarelli Maria Antonietta (A. Renieri)
Creatine transporter defect in males with intellectual disability

Rizzo Valeria (A. Giordano)
New pyrazolo-[3,4-d]-pyrimidine SRC inhibitors induce apoptosis in mesothelioma cell lines through p27 nuclear stabilization

13.00 Closing session and attribution of credits by the faculty board
A copy of the minutes is available at http://www.unisi.it/ricerca/dottorationweb/genetica_medica/ accessing the "Minutes" link.

Introductory speech of the Rector

It is with great pride that I welcome you all to the annual report 2011-2012 day of the new Doctorate in Genetics, Oncology and Clinical Medicine (GenOMeC) of the University of Siena. It is a wonderful occasion to know the results of the research activity of PhD students.

Scientists, especially PhD students, represent the heart of the University Institution. I think that in this historical context the University has to rely on high-quality research and I am confident that this Doctorate will represent a unique strength for the University of Siena.

The GenOMcC Doctorate derives from the fusion between the Doctoral School in Oncology and Genetics coordinated by Prof. Alessandra Renieri and the Doctoral School in Biomedicine and Immunological Sciences coordinated by Prof. Franco Laghi Pasini. This new Doctorate has been evaluated from the Internal Review Board (Nucleo di Valutazione di Ateneo) and it resulted the first of the Biomedical Area and among the top three of the University of Siena. The Internal Review Board was therefore very favorable for the activation of the GenOMeC Doctorate. I would also like to stress that all Doctorates of the University of Siena next year will be subjected to a national evaluation (ANVUR) and only the Doctorates that pass this rating will remain activated.

The Internal Review Board used the ANVUR criteria for evaluation and I am therefore confident that the GenOMeC Doctorate will continue its activity for the coming years.

I want to thank warmheartedly prof. Alessandra Renieri for the past activity in the Direction of Doctoral School and for the commitment to drive the new Doctorate next three years (2011-2014). Finally I want to thank her for the efforts made in fineling external finding resources that are essential at this time for our University.

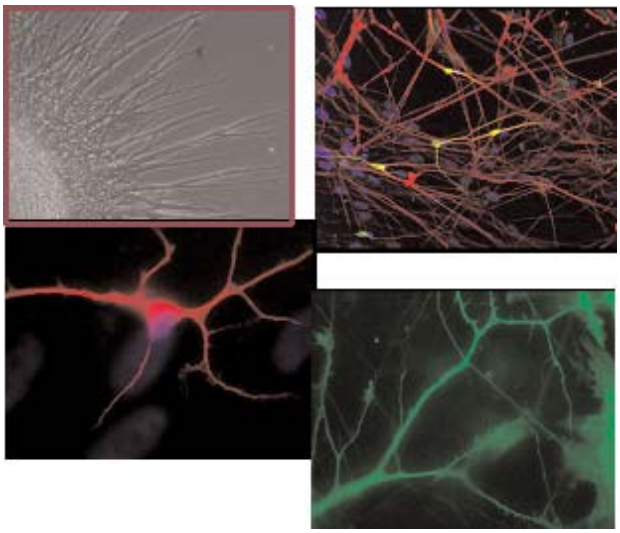
Rector of Siena
Prof. Angelo Riccaboni

Students Project Abstracts

Oncology and Genetics Doctoral School
Medical Genetics
XXIII cycle
Amenduni Mariangela, MS
amenduni2@unisi.it
Tutor A. Renieri

Establishment and validation of a human cellular model for CDKL5-related disorders

Rett syndrome (RTT) is a severe neurodevelopmental disorder almost exclusively affecting females. Due to its incidence (1:10000 born females), it represents the second cause of mental retardation in girls. Mutations in CDKL5 gene have been detected in females with the “early onset seizures variant” of RTT and in males with X-linked infantile spasms. CDKL5 is an highly conserved kinase expressed in a wide variety of cell-lines and tissues, with the highest levels in brain, testes and thymus. The protein is rather uncharacterized but its involvement in RTT has been explained by the fact that this kinase seems to work in a molecular pathway common to that of MeCP2. In spite of intense research efforts, our knowledge of the function of CDKL5 inside the neurons and of the underlying molecular mechanisms, is still limited by the unavailability of both a mouse model and a good human cellular model. To overcome these limitations, we employed the approach of genetic reprogramming that allows the generation of induced pluripotent stem (iPS) cells directly from patients fibroblasts. We successfully reprogrammed fibroblasts from 2 CDKL5-mutated patients (a male with p.T288I and a female with p.Q347X). In order to assess whether iPS cells are suitable as an in vitro model to study the pathogenesis of CDKL5-related disorders, we induced these cells toward a neuronal fate. The characterization of the neuronal differentiation process indicates that iPS cells can be differentiated into neurons and that the differentiation follows the stages observed in human embryonic stem cells (hESCs).Characterization of the obtained neurons is ongoing.



Part of this work is reported in: Amenduni M., et al. iPS cells to model CDKL5-related disorders. Eur J Hum Genet, 2011 Dec; 19(12):1246-55.
This work is funded by: Telethon grants GGP09117 and GTB07001C to A.R.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Disease and Multitumural Syndromes
XXIII cycle
Azzarà Annamaria, MS
azzara@unisi.it
Tutor F. Cetta

Comparison between in Vitro result of Pm incubation with Cell-Lines and Health Effects in children

Both basic and clinical studies have been performed, trying to relate environmental pollution to hospital admissions and/or major respiratory or cardiovascular adverse effects. However, it is always difficult to compare data from “in vitro” studies and experimental models in animals to clinically evident effects in humans.
In the present study, hospital admissions to the main pediatric service of Milan were recorded, analysed and compared with daily and seasonal variation in PM10 and PM2.5 concentration, during 4 consecutive periods: winter 2007- summer 2007- winter 2008- summer 2008. Respiratory diseases were classified as follows: asthma or asthma like disorders; upper respiratory diseases (pharyngitis, pharingotonsillitis, otitis); lower respiratory diseases (bronchitis, bronchiolitis and pneumonia).
During 2007-2008, there were in total 440 pediatric admissions for respiratory diseases; 226 (132 males and 94 females) during the winter semester, and 214 (100 males and 114 females) during the summer semester. There were 12.7% asthma or asthma related admissions; 55.8% due to lower respiratory illness, and 31.5 % due to upper respiratory disease. The daily average of PM10 concentration during the first semester 2007 was 48.3 +/- 17.9 ≒g/m3 median 47. There were 107 (59.1%) days with at least one hospital admission. The mean daily concentration of PM was higher in days with (n=107) than without (n=74) hospital admissions (p=0.032 or <0.05).
In addition, the human bronchial epithelial cell-line BEAS-2B and the human alveolar epithelial cell A549 were seeded at a concentration of 80.000 cell/well and treated after 48 hours with both summer and winter PM10 and PM2.5 sampled in the main Milan urban area. Cytotoxicity was assessed by HOECHST 33342/91 staining. Viability was calculated as the sum of viable mitotic cells. Release of the proinflammatory cytokine-IL was measured by ELISA assay. Oxidative stress was evaluated by chemiluminescence and genotoxicity was assessed by comet assay.
It was found that, whereas A549 cell viability was not significantly reduced after summer and winter PM exposure, summer PM had no significant effects on BEAS-2B viability, whereas winter PM treatment induced a decrease in cell viability, both at the dose of 25 and 50 µg/ml. In addition, whereas both winter and summer PM2.5produced only a slight increase in IL-8 release, winter PM10 induced a 5-fold increase in IL-8 release in treated cells, and summer PM10 induced a 20-fold increase ($p<0,05$) in IL-8 expression.
In particular, BEAS-2B resulted more responsive to PM treatment than A549. Winter PMs were more cytotoxic than summer PMs; Summer PM10 had a higher proinflammatory potential, which could be partly due to biological components (LPS).
Accordingly, acute admission of children was significantly affected by pollution data, with increased admission during winter time, namely for upper air tract infections, whereas lower tract inflammation and acute admission for asthma were more frequent during the spring-summer season.
In conclusion, in vitro studies using PM10 and PM2.5 sampling from different seasonal samples seem to correlate with clinical data in children exposed to the same type and concentration of PM during winter and summer season.
Even if great caution is required when trying to relate in vitro studies to clinical effects in humans, the present report is the first study in a large urban area trying to compare “in vitro” and clinical effects of the same urban particulate material in different seasons of the year.

This work is funded by PROLIFE-Project, Milan, Italy.

Biomedicine and Immunological Sciences Doctoral School
Experimental Rheumatology
XXV cycle
Bertoldi Ilaria, MD
bertoldiilaria@libero.it
Tutor M. Galeazzi

Possible role of hand densitometry in prognostic evaluation and therapeutic management in rheumatoid arthritis. Correlation with instrumental, laboratoristic and clinical index

Bone damage in arthritis appears as periarticular osteoporosis, erosions and generalized osteoporosis. Recently, has been highlighted the central role of osteoclast in the pathogenesis of bone damage in patients affected by arthritis. Osteoclasts' activation is stimulated by a great number of cytokines derived from the synovial and bone marrow environment. The osteoclast activation leads to an excessive bone resorption. Therefore, , in rheumatoid arthritis (RA), in addition to systemic and periarticular osteoporosis, we have to consider the articular osteoporosis, that precedes the erosions' formation, with a relationship of cause and effect. The gold standard for the measurement of bone density is the double-Xray absorptiometry exam (DXA), as confirmed by the WHO guidelines. As demonstrated by recent scientific evidence, DXA can be considered a reliable tool for quantifying bone damage and assessing disease activity, included the RA aptitude to cause bone erosions. Therefore we can use the DXA to monitor therapeutic response. Aim of our work is to measure hand and total body bone mineral density (BMD) of patients at the first diagnosis of RA or with high disease activity that required a new treatment approach. The densitometric values are then correlated with clinic, instrumental and laboratoristic data, in order to evaluate the possible role of bone densitometry in prognosis and in therapeutic assessment of patients affected by RA. We enrolled 10 patients affected by seronegative arthritis and 12 patients affected by rheumatoid arthritis. 12 patients received the first diagnosis and were treated with methotrexate, 10 patient were classified as "non responder" to methotrexate and were treated with an anti-TNF agent. All of them also underwent an ultrasound examination and DXA of the hand. Articular BMD was measured at the most swollen joint, as indicated by the ultrasound examination, with a dedicated region of interest (ROI) created ad hoc for every joint. Inflammatory markers (ESR and CRP) and bone turnover markers were also assessed for all patients. Until now, follow-up visits at three and six months are available for all patients. Data analysis at six months showed a decrease of inflammatory activity, intended as ESR and CRP values and of the disease activity as demonstrated at ultrasonography, whereas half of patients showed stable BMD values and half showed a slight reduction of BMD at the affected joint. To this day, it is not possible to establish the statistical significance of the data regarding the aim of the study. Longer follow-up is required.

Part of this work is reported in: Annual European Congress of Rheumatology EULAR 2010. Rome, Italy, 16 – 19 June 2010. Abstract number: AB0856.

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXIV cycle
Bordignon Matteo, MD
matteo.bordignon@gmail.com
Tutor P. Rottoli

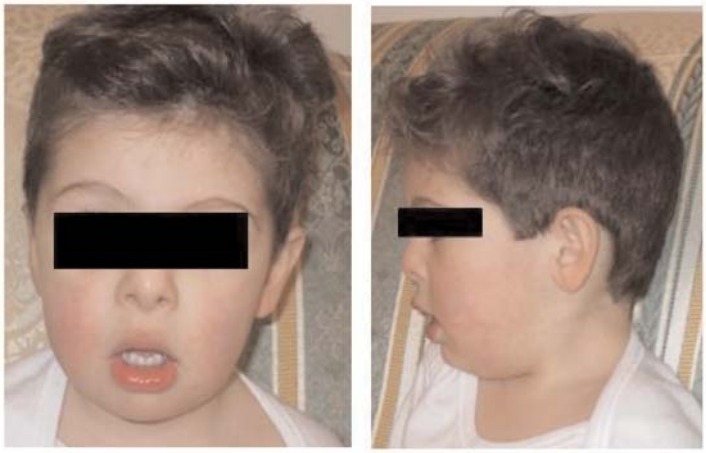
Toll-like Receptor 7 gene polymorphisms analysis in sarcoidosis

Interaction between enviromental triggering factor and pathologic genetic susceptibility towards these factors seems to play a key role in sarcoidosis pathogenesis. Toll-like Receptors (TRLs) are part of the innate immunity and play an essential role in mediating the innate immune response toward microbial pathogens. Recently, a main role of TLRs in autoimmune diseseas was reported. Among TLRs, TLR7 was a susceptibility gene candidate as its role in other immunitary disorders (as Systemic Lupus Erythematosus) has already been reported, it enhances the production of Inteferon and it is localized in chromosome X.
We investigated the “single nucleotide polymorphism” Glu11Leu for TLR-7 gene by TaqMan® assay in 149 sarcoidosis-affected patients and in 151 healty controls.
Among 149 sarcoidosis-affected patients 43% were male and 57% female. Among male patients, 21,9% presented T genotype and 78,1% A genotype. Among female patients, 4,7% presented T/T genotype, 80% A/A genotype and 25,3% A/T genotype.
Among 151 healty controls 43,1% were male and 56,9% female. Among male patients, 18,5% presented T genotype and 81,5% A genotype. Among female patients, 2,3% presented T/T genotype, 55,8% A/A genotype and 41,9% A/T genotype.
Our data shows that T allele is statistically (p=0,0001) low expressed in sarcoidosis-affected patients and consequently that Glu11Leu polymorphism of TRL7 gene may have a protective role in disease development.

Oncology and Genetics Doctoral School
Medical Genetics
XXIV cycle
Bruccheri Maria Grazia, MD
bruccheri@unisi.it
Tutor A. Renieri

A reciprocal translocation t(2;8)(q31;q24) and a de novo 2p22.3p21 interstitial deletion in a patient with developmental delay, joint laxity, peculiar facial features

We describe a 5 years old male patient with mild developmental delay, hypotonia, joint laxity, peculiar facial features and speech delay. Karyotype and FISH analyses revealed a de novo apparently balanced reciprocal translocation involving the long arm of chromosome 2 and 8 [t(2;8)(q31;q24)]. Interestingly, array-CGH analysis identified a de novo interstitial deletion outside the translocation breakpoints, on the short arm of chromosome 2 (del2p22.3-p21). This deletion spans about 8Mb and encompasses 51 genes. Gene content analysis, taking into account gene expression pattern and gene function, pointed out 7 genes of particular interest for the correlation with the patient's phenotype. We compared the clinical features of our patient with two rare cases with overlapping deletions described respectively in the DECIPHER database and in the literature. The first patient presents a smaller deletion 2p22.3-p22.2 spanning about 0.99 Mb, and encompassing 15 genes. The second patient bears a de novo 2p22 deletion associated with a reciprocal translocation (3;7)(p21;q22). There have been only other two reports of interstitial deletions of the short arm of chromosome 2 and none involved band 2p22. This suggests that the deletion identified in our patient causes a distinct clinical phenotype, not previously described. Furthermore, this report, confirms previous data showing that apparently balanced translocations often hide complex chromosomal rearrangements.



Biomedicine and Immunological Sciences Doctoral School
Osteo-Metabolic Diseases
XXIV cycle
Cadirni Alice, MS
Tutor R. Nuti

The impact of BMI and body composition on circulating levels of OPG, RANKL, MGP, NT-proCNP in type 2 diabetic patients and in normal subjects

Recent observations have suggested that several cytokines involved in bone formation may also be implicated in diabetic vasculopathy. Among these osteoprogeterin (OPG)/RANKL/RANK axis, vitamin K dependent matrix GLA protein (MGP) and C-type natriuretic peptide (CNP) have been reported to play an important role. This study aimed to evaluate the impact of BMI and body composition parameters on the serum levels of OPG, RANKL, MGP and NT-proCNP in normal and subjects with type 2 diabetes (DM2).
In 150 DM2 patients and 190 controls OPG, RANKL, MGP and NT proCNP were measured. Intima media thickness and carotid plaque echogenicity were assessed by ultrasonography. The body composition was performed by DXA.
MGP was significantly lower in the diabetic population than in the normal subjects (7,39 ± 2,2 nmol/l vs 12,9 ± 6,4 nmol/l). In both DM2 and control subjects MGP was inversely associated with carotid stenosis ($r = -0,15$; $p < 0,05$ and $r = -0,17$; $p < 0,05$). A significant correlation between MGP and IMT ($r = -0,17$; $p < 0,05$) was found in diabetics. MGP was directly correlated with BMI, total fat mass and abdominal fat mass ($p<0.01$), whereas in controls MGP was inversely correlated with fat percentage ($p<0.05$) .
OPG levels were higher in DM2 patients than in normals and associated with atherosclerosis. OPG was also correlated with HbA1c ($p<0.05$).
NT-proCNP was lower in diabetic subjects ($p< 0,05$). A direct correlation was found between carotid calcification and NT-proCNP in both normals and diabetics ($p <0,05$). In controls but not in DM2 patients NT-proCNP was positively correlated with BMI.
In conclusion, our results seem to confirm that OPG, MGP and NT-proCNP play a role in the carotid atherosclerosis in both DM2 and control subjects.
The influence of BMI and body composition on MGP and CNP differs between diabetic and non diabetic subjects.

Biomedicine and Immunological Sciences Doctoral School
Experimental Medicine and Atherosclerosis
XXVI cycle
Cappelli Alessia, MS
cappelli23@unisi.it
Tutor P. Tanganelli

ALTERATION OF AORTIC CONSTITUENTS IN THORACIC AORTIC ANEURYSM AND ROLE OF MMPs, TIMPs AND GGT IN THE PATHOGENESIS OF THE DISEASE

Aneurysmal disease of the aorta, defined as a focal dilation exceeding 150% of the normal arterial diameter, is a potentially fatal disease occurring with increasing frequency in our aging population. Aneurysm can affect the thoracic and abdominal aorta, whose pathogenesis appear to be different. We focused the attention on aneurysm of ascending thoracic aorta. Thoracic aortic aneurysms occur when the arterial wall is unable to resist the dilating force of arterial pressure. This has been attributed primarily to mechanical failure of one or more of its major structural components, notably collagen and elastin. However, recent research shows that dilation of aorta depends upon complex biological factor, including increased proteolitic activity in aortic wall by metalloproteinase (MMPs). Also the role of gamma-glutamyltransferase (GGT) in recycling glutathione seems to be important for the maintenance of cellular homeostasis and protection from oxidative damage. At the same time, the release of reactive compounds resulting from this process, such as cysteinyl-glycin, is responsible of hydroxyl radicals production and pro-oxidant extracellular environment generation.
The aim of this study is to investigate the morphological changes of the aortic wall, the role of the MMPs and their inhibitors TIMPs in the development of aortic aneurysm and to evaluate tissue localization and plasma concentration of GGT to establish a possible involvement of this protein in the pathogenesis of thoracic aortic aneurysm.
We collect 45 samples that are subdivided in two groups: true aneurysm and annulo-ectasia (where the dilatation also involves the annulus). We make a comparison between the two groups to evaluate the involvement of proteins in the two different expression of the disease.
Histological examination has been completed and showed that the disease involves primarily the media layer. The main tissue alterations are the following: focal depletion of smooth muscle cells, especially in the middle and inner third of the media; substantial decrease of smooth muscle cells, mainly in the area underlying the large plaques; sometimes, focal calcifications of isolated smooth muscle cells and elastic laminae.
Atherosclerosis compares in many samples essentially in the form of sclero-atheromatous plaques with atheroma and thick top cap; sometimes we observe sclero-calcific plaques.
The interstitial matrix shows increase of mucopolysaccharides, especially in the middle and inner third of the media. Sometimes, the accumulation of polysaccharide material leads to the formation of "lakes" or "pooling" and "cystic areas."
We observe vasa vasorum that originate from adventitia and creep into the media. There are also neovascularization areas that extend beyond the outer third of the media, occasionally reaching up to the third subintimal layer, near plaques.
Lymphocytic infiltrates has a variable degree and localizes around vasa vasorum.
The study of MMPs and TIMPs revealed that aneurysm disease is the consequence of a dysregulated balance between matrix degradation and matrix deposition process, primarily mediated by the altered and abundance expression of MMPs TIMPs.

Oncology and Genetics Doctoral School
Oncological Genetics
XXVI cycle
Casini Nadia, MS
casini12@unisi.it
Tutor A. Giordano

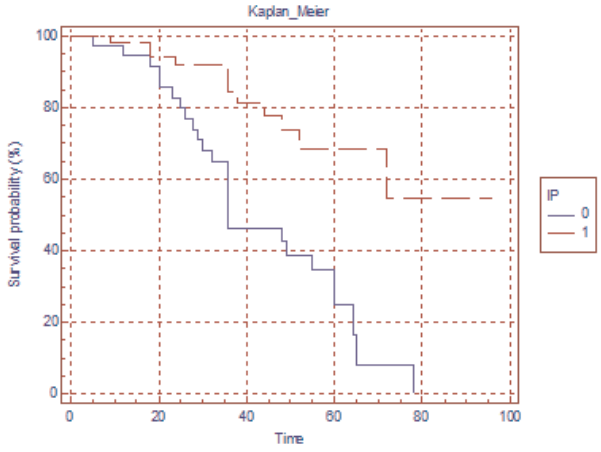
Role of the Cdk9/Cyc complex during muscular differentiation in normal and pathological conditions

Cyclin-dependent kinase 9 (Cdk9) is a serine-threonine kinase involved in many cellular processes. As other Cdks, Cdk9 needs to bind to a cyclin (cyclin T or K) forming the P-TEFb complex. Cdk9 exists in two isoforms: Cdk9-42 and Cdk9-55. Both phosphorylate the carboxi-terminal domain (CTD) of RNApolII, but their roles are different from cell to cell. The purpose of the present study is to investigate the role of Cdk9-55 during skeletal muscle differentiation and regeneration.
The results show how the two isoforms are differently regulated during differentiation and regeneration. We firstly treated wild type mouses with CTX (cardiotoxin), disrupting skeletal muscle and then we harvested muscles in regeneration after different times post treatment. We found that Cdk9-55 is strongly upregulated during regeneration. On the contrary Cdk9-42 levels do not change during regeneration, suggesting a major involvement of Cdk9-55. We also analyzed the expression of MyoD and Myf5, two muscular transcriptional factors which bind Cdk9. As expected there is an upregulation of MyoD and Myf5, indicating that the skeletal muscle is undergoing differentiation and regeneration.
To investigate how the two isoforms are modulated during skeletal muscle differentiation we analyze different samples: mouse embryos, limbs cultures and L6E9 and C2C12 cell lines. Mouse embryos were collected at different times of development. They show a different pattern of expression. Cdk9-42 is upregulated in the first stages of development, while Cdk9-55 in more expressed in the latter, suggesting a different role of the two isoforms. At the same times embryo limbs were harvested and cultured. Furthermore they were induced to differentiate and then collected. Cdk9-55 is much more expressed during differentiation in spite of the expression of Cdk9-42. Then L6E9 and C2C12 were cultured and differentiated respectively with BSA and HS (horse serum). The results show how in both cell lines Cdk9-55 is upregulated as the differentiation process progress and Cdk9-42 in downregulated. These results suggest a major involvement of Cdk9-55 during skeletal muscle differentiation.
Further studies were focused on the understanding of which cyclin partner could associate with the two isoforms. Emerging evidence suggest that cyclin K could associate with Cdk9-55 to activate the differentiation process. The expression of cyclin K is strongly upregulated as differentiation begin.
In the future we will investigate the role of Cdk9 in rhabdomyosarcoma cell and which cyclin is involved. Furthermore we will evaluate the ability of the Cdk9/cyclin complex to bind MyoD, a transcriptional factor responsible for the formation of the skeletal muscle fibers.

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXVI cycle
Castria Donatella, MD
donatellacastria@yahoo.it
Tutor P. Rottoli

Pulmonary hypertension in idiopathic pulmonary fibrosis – prevalence and clinical progress

The present study aimed to define the prevalence of Pulmonary hypertension (PH) in a numerous cohort of Idiopathic pulmonary fibrosis (IPF) patients, to investigate the correlations between systolic pulmonary artery pressure (PAPs) and functional data, to evaluate clinical progress and to compare long-term survival in IPF patients with and without PH.
A population of 126 patients affected by IPF was recruited. High prevalence of PH (39.7%, 50/126) (PAPs > 36 mmHg evaluated by echocardiography) was observed in this population , mainly in smokers and female patients. When PH patients were divided into two subgroups (PAPs echocardiography greater or less than 50 mmHg), regression analysis revealed a significant correlation between PAPs > 50 mmHg and DLCO/VA (p=0.029). Mean PAPs values significantly increased after 1 year from the clinical onset of PH (p=0,01). 11/21 patients with FVC <50% had a significant increase of PAPs values after 1 year from PH onset (p=0,02). There was a highly significant difference between survival in IPF patients with or without PH (p=0.0001; hazard ratio = 3.56).
This study confirmed that PH has a high prevalence in patients with IPF and it is associated with increased mortality risk. Early PH diagnosis is strongly important: PH is associated with high mortality in IPF, therefore these patients should be quickly insert in waiting list for lung transplant.

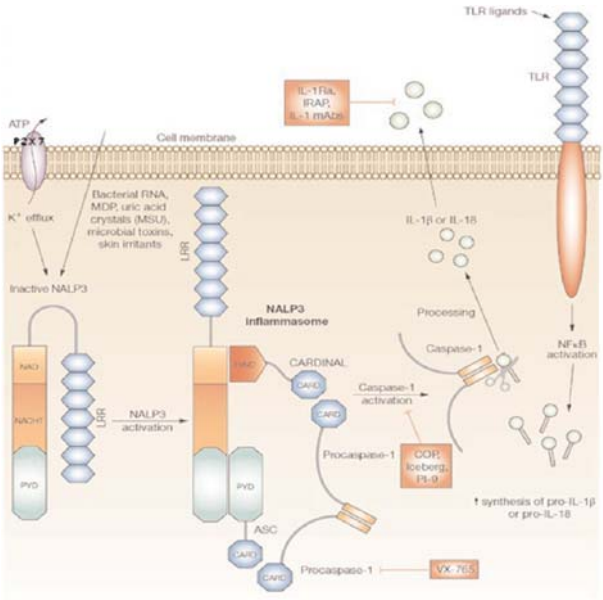


Part of this work is reported in:
- Ipertensione Polmonare e Fibrosi Polmonare Idiopatica: andamento clinico e approccio terapeutico; National Congress of Pneumology UIP, Milan 2010: Poster.

Biomedicine and Immunological Sciences Doctoral School
Clinical Pharmacology
XXIV cycle
Castrichini Monica, MD
Tutor F. Laghi Pasini

Expression and function of the P2X7 purinergic receptor in peripheral blood mononuclear cells from patients with Behçet's disease

P2X7 receptor is a nucleotide-gated ion channel chiefly involved in the inflammatory response triggered by passive release of ATP from damaged cells. It is largely expressed in monocytes and plays a key role in promoting the release of pro-inflammatory cytokines, particularly IL-1β. Behçet's disease (BD) is a systemic immune-inflammatory disorder of unknown origin whose clinical manifestations include oral and genital ulcers, skin lesions, uveitis and arthritis. Since innate immunity activation and IL-1β release seem to play a relevant role in BD, we hypothesized a P2X7 involvement in the pathogenesis of the disease. On this basis, we evaluated the expression and function of P2X7 receptor in the peripheral blood mononuclear cells (PBMC) from BD patients.
PBMC, or isolated monocytes, were prepared from 18 BD patients and 17 healthy controls matched for age and sex. In these cells we evaluated: P2X7 expression and function, induced by P2X7 receptor stimulation, as determined by cytosolic free Ca2+ fluxes measurements, IL-1β release and apoptosis induction. In BD monocytes P2X7expression and the amount of Ca2+ influx, induced by the selective P2X7 receptor agonist 2'-3'-O-(4-benzoylbenzoyl)ATP (BzATP) were higher than in monocytes from healthy controls. Moreover, in BD patients BzATP stimulation significantly enhanced the release of IL-1β from lipopolysaccharides (LPS)-primed monocytes, and promoted apoptosis in PBMC. Our results provide evidence that in PBMCs from BD patients both the expression and the function of the purinergic P2X7 receptor are increased with respect to healthy controls. These data, suggesting the putative involvement of this pathway in the pathogenesis of the immuno-inflammatory activation underlying BD, may designate the P2X7 receptor as a new potential therapeutic target of the disease.



Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXV cycle
Cervadoro Elisa, MD
elisacervadoro@inwind.it
Tutor M.Fimiani

Psoriasis as systemic desease: metabolic syndrome and celiac disease like prognostic factors

The project is carried out by inserting into the wider national and PSoCare Project, Project PSODIT then converted, in particular, have been selected a subgroup of 203 patients with psoriasis (71 women and 132 men), which refer to the centers of PSoCare respectively UODermatologia Siena (University), Department of Dermatology of Pisa (University) and Dermatology Unit of Livorno (Hospital). Patients are selected from those included in the Project PSOC / Psodit. Patients are required for laboratory analysis for the monitoring of certain interleukins and cytokines: IL-1, TNF-alpha INFgamma, IL-12,, IL-6 and Il- 8, IL-10, ESR, CRP, ANA. In fact for some years has been established a close relationship between S.me metabolic and rheumatic diseases, primarily inflammatory arthritis and arthropathy. And 'demonstrated a mortality rate from cardiovascular causes by 36% in patients with psoriatic arthritis with an incidence of about 1.3 times higher than the general population.
TNF-alpha, produced by adipocytes under conditions of insulin resistance and fat cell hypertrophy, causing processes infiammazione.Inoltre IL-6, released from adipose tissue into biologically more quantities as in the case of obese patients and in ' hepatic insulin resistance, causes the activation of inflammatory processes. In addition, there is undisputed evidence that inflammation is at the base of the atherosclerotic process and that the C-reactive protein (CRP) is an independent risk factor for cardiovascular disease, related to insulin resistance, together with a greater expression of IL- 1b, IL RA -1, IL-6, IL-8. In addition to the reduction of the role of anti-infiammmatorio HDL-apo AI that have the ability to block the contact between activated lymphocytes and monocytes, thereby inhibiting the production of TNF-a and IL-1b.TNF-alpha, produced by adipocytes under conditions of insulin resistance and fat cell hypertrophy, causing processes infiammazione.Inoltre IL-6, released from adipose tissue into biologically more quantities as in the case of obese patients and in ' hepatic insulin resistance, causes the activation of inflammatory processes. In addition, there is undisputed evidence that inflammation is at the base of the atherosclerotic process and that the C-reactive protein (CRP) is an independent risk factor for cardiovascular disease, related to insulin resistance, together with a greater expression of IL- 1b, IL RA -1, IL-6, IL-8. In addition to the reduction of the role of anti-infiammmatorio HDL-apo AI that have the ability to block the contact between activated lymphocytes and monocytes, thereby inhibiting the production of TNF-a and IL-1b.
Some studies indicate a high incidence of overt or silent celiac disease in patients with psoriasis. The antigliadin antibodies are more common in patients with psoriasis in the general population. Studies show that a gluten-free diet improves psoriasis lesions. The T lymphocytes in patients with celiac disease similar to release cytokines IFN-γ and IL-2 produced in patients with psoriasis. In the study I have that cell proliferation and trans-glutaminasi tissue in the skin of patients affected with psoriasis, before and after 3 months of gluten-free diet, showed a significant reduction in cell proliferation, after the diet, even in patients without an increase intraepithelial lymphocytes. After 3 months of gluten-free diet, tissue transglutaminase has decreased by 50%.

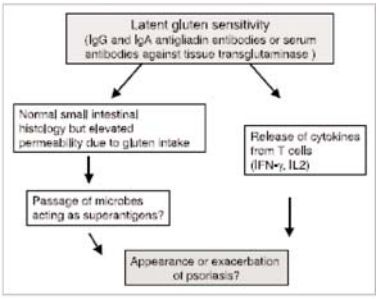


Fig 2. Potential effects of 'silent' coeliac disease. IFN, interferon; IL, interleukin.

Part of this work is reported in: Psoriasis today: managment of patients and therapeutic approacces, Livorno, 29 October 2011.

Oncology and Genetic Doctoral School
Medical Genetics
XXIV cycle
Colecchia David, MS
colecchia2@unisi.it
Tutor M. Chiariello

Involvement of the Erk8 in autophagy

Autophagy is an evolutionarily conserved lysosomal self-digestion process involved in degradation of long-lived proteins and damaged organelles. Autophagy is activated in cancer cells in response to multiple stresses and has been demonstrated to promote tumor cell survival and drug resistance. Likewise, it has been extensively reported that autophagy functions also as a tumor suppressor mechanism. Hence, the precise role of autophagy during cancer progression and treatment is both tissue and context dependent.
In a yeast two-hybrid screening, we have identified multiple positive clones, encoding for GABARAP and GABARAP-L1, two well-known proteins acting in autophagic process, able to interact with ERK8. We confirmed these interactions in vivo and in vitro using multiple biochemical assays as affinity purification and co-immunoprecipitation. Using ERK8 deletion mutants, we also identified the domain responsible for the interaction with GABARAP and GABARAP-L1 in the region included between 273 and 373 aminoacids.
Furthermore, as GABARAP and GABARAP-L1 belongs to LC3 protein family of autophagic modifiers we valuate also the interaction between ERK8 and LC3B, the main autophagic marker. We confirmed such interaction and observed that region between 272 and 373 aminoacids is required for ERK8 to bind LC3B. As autophagic modifiers locate on autophagic vesicles during autophagy, we analyzed ERK8 localization. ERK8 displays a vesicular pattern, and co-localizes with LC3B and GABARAP in autophagic structures.
Up-to-date little is know of ERK8 activation pathway in comparison to canonical MAP kinases, thus the simplest way to analyzed ERK8 downstream effect is its over-expression. In our cellular model, an increase of ERK8 leads to an up-regulation of LC3B turnover, a typical hallmark of autophagy induction. Moreover, ERK8 co-localizes in autophagosomes with p62, a wide-used marker of autophagy; over-expression of ERK8 increase of the amount of p62-positives vesicles in presence of Bafilomycin A, an inhibitor of autophagic degradation.
Altogether, these data suggest a potential involvement of ERK8 in regulation of autophagy. Recently, ERK8 has been involved in cancer processes (Henrich, 2003, Mol Cell Biol; Xu, 2010, Cancer Res; Rossi, 2010, JBC). Hopefully, elucidating ERK8 role in autophagy may clarify also its role in cancer.

Part of this work is reported in: Rossi M. et al J Biol Chem 2011Mar 11; 286(10): 8507-22
This work is funded by: Start-up grant from Istituto Toscano Tumori to Mario Chiariello (2007-2011)

Oncology and Genetics Doctoral School
Oncological Genetics
XXIV cycle
Conti Daniele, MS
conti44@unisi.it
Tutor A. Giordano

Small molecules mimicking the spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents

Over the past decades, cancer research has been mainly aimed at identifying the molecular alterations underlying cancer development, in order to design new drugs for targeted therapy.
The proteins of the retinoblastoma family, consisting of pRB1/p105, pRB2/p130 and pRBL1/p107, are of great interest because of their tumor suppressor activity. We focused in particular on pRB2/p130, the expression of which is altered in many cancer, in order to identify strategies that could restore its tumor suppressor function. pRB2/p130 is able to bind the complex formed by Cyclin A and the Cyclin-dependent kinase CDK2, causing the inhibition of its kinase activity, which is necessary for cell cycle progression. pRB2/p130 consists of two highly conserved regions, A and B, which are separated by a spacer domain to which has been attributed its inhibitory effect on Cdk2/Cyclin A activity, in particular to a 39 aa-long spacer-derived peptide (Spa310, aa 641-679).
We used a computational chemistry approach to select a pool of small molecules that mimic Spa310 activity. The analysis of the CDK2-CyclinA crystal structure allowed us to select five hypothetical CDK2-CyclinA inhibitors from chemical libraries. We tested the antiproliferative effects of these five small molecules on cell lines of different tumor types (lung and prostate cancer, osteosarcoma, mesothelioma and medulloblastoma) by the MTS cytotoxicity assay. We observed a significant reduction in the growth rate of these tumor cells and we focused our further analyses on the most effective compound. In order to rule out the potential cytotoxic effect on normal cells, we tested these molecules also on non-neoplastic cell lines, finding that they have a significant minor effect on normal cells with respect to their tumoral counterpart. Consequently, we performed FACS analysis and caspase-3 assays in order to verify the activation of apoptosis in the treated cancer cells and we confirmed that the selected small molecule is able to induce apoptosis in lung cancer cell lines.
To dissect the molecular mechanisms by which our compound exerts its inhibitory activity, we performed a kinase assay showing that inhibition of kinase activity, and thus of phosphorylation, was observed only when using GST-Rb2-Spacer as substrate of the reaction while no reduction of activity was observed on the control substrate. Additionally, in order to verify the specificity of our compound for inhibition of CDK2-Cyclin A only, we also tested it on a small panel of ser-treonine kinases which were not affected by the presence of our molecule. Immunoblotting of whole cell lysate prepared from lung cancer cells treated with our compound showed inhibition of Rb2 phosphorylation with levels of non-phosphorylated Rb2 remaining unaffected. These data taken together confirm not only that our molecule is a non-ATP competitor inhibitor of CDK2-CyclinA kinase, but also that it is a highly selective inhibitor of Rb2 phosphorylation.
As a future objective, we intend to test these small molecules in mouse tumor xenografts in order to evaluate their ability to inhibit tumor growth also in vivo.

Part of this work is reported in: Small molecules mimicking the spa310 peptide from the spacer region of pRB2/p130 as potential anticancer agents. Fifth Annual Scientific Conference – Istituto Toscano Tumori (ITT), Il Borro, San Giustino Valdarno (Arezzo), July 1, 2010.

This work is funded by: Istituto Toscano Tumori (ITT) and Human Health Foundation (HHF).

Biomedicine and Immunological Sciences Doctoral School
Osteo-Metabolic Diseases
XXVI Cycle
Corallo Claudio, MS
corallo@unisi.it
Tutor N. Giordano

Potential Benefits of the new Therapeutic Application of Musically Modulated Electromagnetic Fields (TAMMEF) in Human Primary Osteoarthritic Chondrocytes

Osteoarthritis (OA) is the most frequently occurring rheumatic disease, caused by metabolic changes in chondrocytes, the cells that maintain cartilage. Literature reported that treatment with electromagnetic fields (EMFs) produces benefits in patients affected by this pathology .Isolated human primary osteoarthritic (OA) chondrocytes were cultured in vitro under special conditions in order to mimic the imbalance between chondroformation and chondroresorption processes observed in OA cartilage in vivo. The cells were exposed for a specific time (30 min/day for 15 days) to extremely low frequency (ELF) electromagnetic fields with fixed frequency of 100 Hz and to the Therapeutic Application of Musically Modulated Electromagnetic Fields (TAMMEF), which are characterized by variable frequencies, intensities, and waveforms (always in the range of extremely low frequencies). We tested the effects of the different types of exposure on chondrocyte metabolism. The exposure of the cells to TAMMEF system enhances cell viability and proliferation, does not generate reactive oxygen species, does not cause glutathione depletion or changes in mitochondrial transmembrane potential compared to cells exposed to ELF system. Moreover, electron microscopy analysis (TEM) showed regular morphology of cells treated with TAMMEF system compared to cells treated with ELF system that showed phenomena of apoptosis and necrosis characterized by a large numbers of autophagic vesicles (AV) and irregular nuclei inside. This study suggests that EMFs in the range of extremely low frequencies (ELF) positively influence the vitality of OA chondrocytes if the right set of codes (a combination of frequencies, intensities and waveforms) is caught by cells. In conclusion, TAMMEF system can be recommended for OA therapy and represents a valid non-pharmacological approach to the treatment of this pathology.

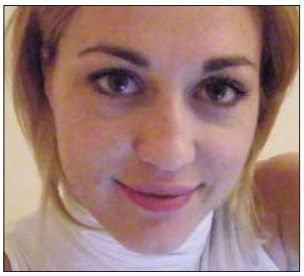
This work is supported by: TAMMEF Centre, University of Siena, Siena, Italy

Oncology and Genetics Doctoral School
Oncological Genetics
XXV cycle
Cozzi Martina, MS
cozzi@unisi.it
Tutor A. Giordano

New small molecule inhibitors of SRC as potential candidates for cancer therapy

There is growing evidence that alterations in the activity of the tyrosine kinase SRC play a key role in the development and progression of several human cancers. SRC has been shown to be an important molecular target in cancer therapy.
This study aims at investigating the effects of new pyrimidine derivative SRC inhibitors in a panel of tumors that show a high SRC kinase activity. Given the central role of SRC in regulating several key processes in tumor development, we plan to analyze the effects of the SRC inhibitors on these processes.
The first part of the project has been devoted to the study of the effects of these molecules in medulloblastoma, the most common malignant brain tumor in children. Despite improvements in the overall survival rate, it still lacks an effective treatment. A high SRC activity was identified in medulloblastoma, suggesting that SRC could have a key role in the development of this tumor. We have examined the effects of the SRC inhibitors in human medulloblastoma cell lines (Daoy and D283) and showed that these inhibitors greatly reduce the growth rate of medulloblastoma cells compared with a non-neoplastic nerve cell line (HT22). These compounds halt cells in the G2/M phase, and this effect likely occurs through the regulation of CDK1 and CDC25C. Moreover, the exposure to pyrimidine derivatives induces apoptosis through modulation of the apoptotic proteins BAX and BCL2, and inhibits tumor growth in a xenograft mouse model. Notably, the small molecules show major inhibitory effects on medulloblastoma cell growth compared with the chemotherapeutic agents cisplatin and etoposide. In conclusion, our results suggest that SRC ihibitors could be novel attractive candidates for the treatment of medulloblastoma.
Recently, we tested the effects of the SRC inhibitors also in Burkitt's lymphoma (BL) cell lines. BL is a highly aggressive and rapidly proliferating B cell neoplasm, which accounts for 30-50% of lymphomas in children and 1-2% in adults. Although intensive polychemotherapy regimens has proven very effective, they are associated with significant toxicities. Therefore, more rationale therapies that selectively target the molecular abnormalities of BL are needed. Recent data suggest that SRC could represent an important therapeutic target for BL. We found that our SRC inhibitors exerted a significant cytotoxic effect and induced apoptosis on two BL cell lines, as determined by MTS assays, cytofluorimetric analyses and caspase-3 assay. Notably, our SRC inhibitors proved to be more effective than the well-known SRC inhibitor PP2 in BL cells. We also observed that our small molecules induced a G2/M arrest in BL cells and, by western blotting analyses, we identified a possible new mechanism, dependent on changes in the activity of the kinases AKT, WEE1 and the CDK1, by which SRC inhibition can induce this growth arrest.

This work is funded by:
U.S. National Institutes of Health grants and by the Sbarro Health Research Organization (http://www.shro.org), the Human Health Foundation Onlus Spoleto, Italy (http://www.hhfonlus.org), and the Teresa and Luigi de Beaumont Bonelli Foundation (to A.G.).



Oncology and Genetics Doctoral School
Colorectal and gastroesophageal diseases
XXIV cycle
Crucianelli Francesca, MS
crucianelli@unisi.it
Tutor S. Civitelli, M. Genuardi

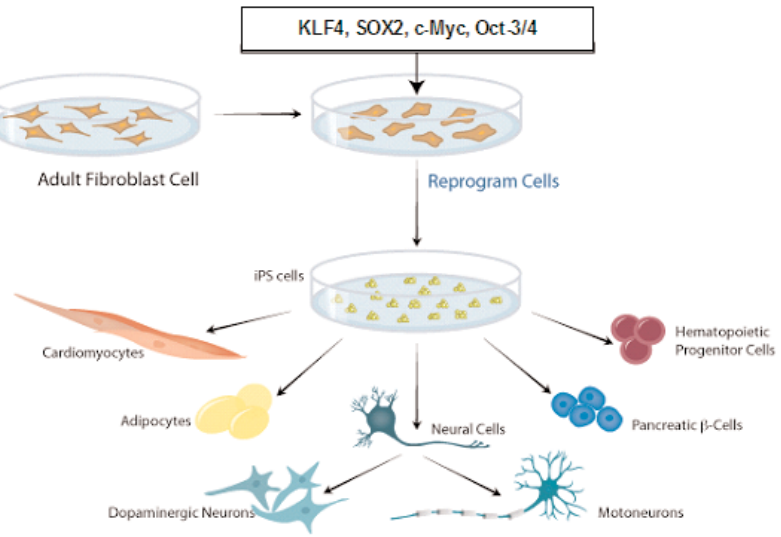
Analysis of constitutional epigenetic changes in colorectal cancer and in multiple primary tumors (MPT) by MS-MLPA technique

Aberrant methylation of CpG-islands has been shown to be associated with transcriptional inactivation of tumor suppressor genes in a wide spectrum of human cancers. Evidences show that germline epimutations of DNA repair genes can cause cancer predisposition. Recent studies provide a proof-of-concept that gene promoter methylation is associated with tumor multiplicity.
This project focuses on the investigation of aberrant constitutional DNA methylation in mutation-negative patients who are suspected to be at high risk of cancer. The study is being developed by analyzing two different groups of patients: a)subjects with multiple primary tumors (MPT); b)subjects with clinical suspicious of Lynch syndrome. The search has been performed on non cancerous tissues. In addition, we have analyzed tumor tissue, when available. Methylation analysis has been performed by a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay. Two different kits of MS-MLPA has been used: one kit allows detection of CpG methylation in 24 different tumor suppressor genes, the other one allows analysis of CpG methylation status of 7 MMR genes. Recently, we have developed a Methylation-Specific PCR (MS-PCR) to assess methylation status of MLH1 promoter region.
So far, 44 patients with MPT and 9 patients with clinical suspicious of Lynch syndrome, have been investigated. For seven of these latter, tumors were available to compare methylation patterns. No alteration of MS-MLPA peak ratios has been observed in this small subset of patients.

Oncology and Genetics Doctoral School
Medical Genetics
XXIII cycle
De Filippis Roberta, MS
Tutor A. Renieri

FOXG1 and Rett Syndrome: functional characterization and set-up of an in vitro human cellular model

FOXG1 gene encodes for a fork-head box protein G1, a transcription factor acting primarily as transcriptional repressor through DNA binding. In 2007, by array-CGH analysis in patients with Rett-like phenotype, we identified FOXG1 as the third gene responsible for Rett Syndrome (RTT). To clarify the clinical phenotype of FOXG1 mutated patients, we performed molecular analysis of the gene including an international cohort of patients and 4 new cases were identified.
At the cellular level, FOXG1 is localized in the nucleus and it is dynamically involved in global chromatin regulation. Functional characterization of the protein revealed that FOXG1 binding to chromatin is reversible even if a significant fraction of the total protein is stably bound. During early neurodevelopment, FOXG1 is essential for the correct neurogenesis of the telencephalic progenitors cells. However, the protein is still expressed in post natal tissue suggesting that its function could be essential also in post-mitotic neurons. Recently, promising results for the study of neurodevelopmental disorders have been obtained by the application of iPS technology. To investigate FOXG1 function at neuronal level, we thus set up the reprogramming protocol in our laboratory in order to obtain iPS cells starting from fibroblasts isolated from FOXG1-mutated patients with the final aim to differentiate them into neurons. This approach gives the opportunity to obtain in vitro affected neurons from a specific individual bypassing all legal and ethic limitations. Moreover, this approach open the way to patient-specific drug screening since obtained cells are genetically identical to the patient from whom they have been generated.



This work is founded by:
Telethon grants GTB07001 and GDP09117 to A. R.

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXIV cycle
De Santis Sante, MD
Tutor D. Nuti

Ghrelin, leptin and proinflammatory cytokine levels in patients with Obstructive Sleep Apnea-Hypopnea Syndrome (OSAHS)

Aim of this study was to investigate the relationship between the severity of sleep apnea and body mass index (BMI) with plasma levels of leptin, ghrelin, interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). To compare the independent contribution of sleep apnea and obesity to plasma levels of leptin, ghrelin, IL-6 and TNF-α, we investigate their serum concentrations in 20 obese (BMI > 30 Kg/m2) and 6 non-obese patients (BMI < 30 kg/m2) with OSAHS. According to Apnea-Hypopnea Index (AHI), OSAHS was classified as: severe (AHI > 30), moderate (AHI: 15-30) and mild (AHI < 15). Twelve patients were treated with nasal continuous positive airway pressure (nCPAP) and 14 with surgery. Each patient was submitted to Polisomnography (PSG) and to ENT examination with flexible endoscopy and Muller maneuver. Fasting leptin, ghrelyn and cytokine levels were measured at baseline and 2 days and 2 months after initiation of nCPAP treatment and 6 months after surgery, respectively. Baseline leptin and ghrelin levels were higher in the moderate to severe OSAS patients than in the mild OSAS group. There was also a significant positive correlation between leptin and BMI. The ghrelin serum levels were significantly lower in obese patient. Serum IL-6 and TNF-α levels were correlated with AHI , but there was no significant difference between obese e non-obese patient. Leptin, ghrelin and cytokine levels did not change significantly from baseline after 2 days of nCPAP while the values decreased significantly, although the BMI of the patients showed no change, after 2 months. After 6 months, postoperative leptin, ghrelyn and cytokine values were significantly lower in 14 patients underwent surgical treatment. These preliminary data indicate that elevated leptin and ghrelyn levels are not determined by obesity alone, since they decreased with AHI reduction. Our results demonstrated, also, significantly higher proinflammatory cytokine levels in patients with OSAHS, not correlated with variation of BMI. In conclusion , this study suggests that blood leptin, ghrelyn, IL-6 and TNF-α levels may represent important markers in the assessment of OSAHS . They are independent factors regards obesity and BMI.

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXV cycle
Defina Marzia, MD
defina2@unisi.it
Tutor M. Bocchia, F. Lauria

Identification of a novel p190-derived breakpoint peptide suitable for peptide vaccine therapeutic approach in ph+ acute lymphoblastic leukemia patients

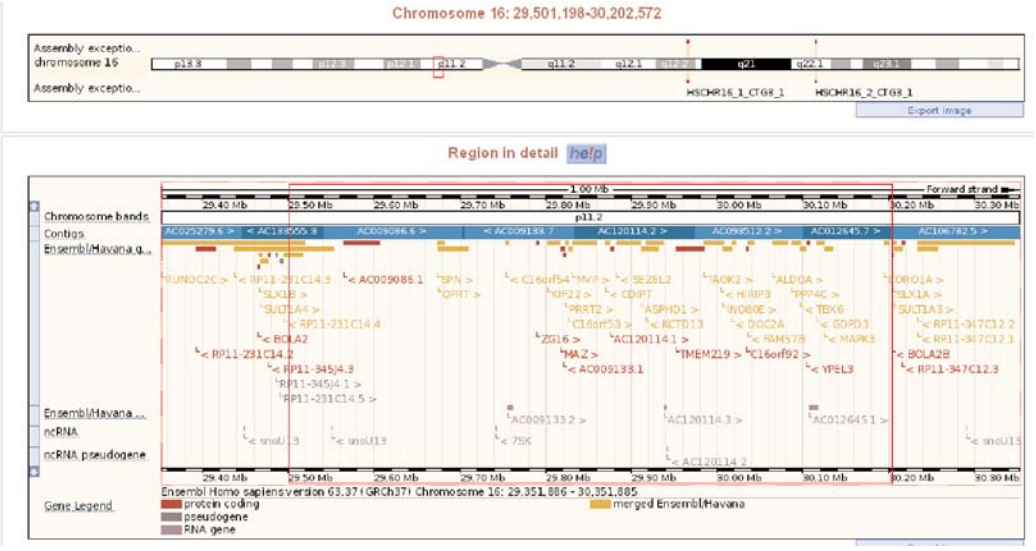
Ph+ acute lymphoblastic leukemia (Ph+ALL) is a high-risk acute leukemia with poor prognosis, in which the specific t(9;22)(q34;q11) translocation results in a chimeric bcr-abl (e1a2 breakpoint) and in a 190 KD protein (p190) with constitutive tyrosine kinase activity. The advent of first and second generation tyrosine kinase inhibitors (TKIs) improved the short term outcome of Ph+ALL patients not eligible for allo-SCT; yet disease recurrence is almost inevitable. Peptides derived from p190-breakpoint area are leukemia-specific antigens that may mediate an antitumor response toward p190+ leukemia cells. Of note, Riva et al. (Blood,2010) have recently found “natural” BCR-ABL breakpoint specific cytotoxic T lymphocytes (CTLs) in the bone marrow of Ph+ ALL patients treated with imatinib correlating with a better response to this TKI. These recent evidences together with the encouraging results observed with therapeutic p210 derived peptide vaccine for CML reinforced the rationale of developing a breakpoint peptide vaccine in Ph+ ALL patients as immune target therapy synergistic with TKIs. In particular, we searched for p190 breakpoint peptides strongly inducers of a peptide-specific CD4+ T cell response, yet including in their sequences many class I epitopes for CTLs response.
We employed standard peptide-HLA binding databases to investigate the p190 fusion region for novel 25mer peptides with strong HLA class II binding prediction and with class I epitopes in their sequence. All promising peptides were synthesized and purified by HPLC for in vitro use. The capability of identified peptides to induce peptide-specific CD4+ T cells was performed with CD4+ T cells freshly isolated from PBMC. CD4+ cells were cultured for 21 with autologous CD14+ cells and different peptides in the presence of IL-15.
Three promising 25mer long fusion peptides named p190-13, p190-15 and p190-17 were identified with strong HLA binding properties for HLA-DRB1*0101, HLA-DRB1*0401, HLA-DRB1*1101 and HLA-DRB1*0301(DR17). Only p190-13 peptide (PDNGEGAFHGDAEALQRPVASDFEP) was able to induce peptide specific CD4+ T cell proliferation in all 5 healthy donors tested as measured by standard 3HThymidine assay, with a stimulation index (SI) ranging from 2.0 to 2.5 (SI= cpm CD4+ T cells + test peptides/CD4+ T cells + control peptides; proliferation was considered positive for SI≥2). We tested p190-13 immunogenicity also in 2 Ph+ALL patients in complete remission during imatinib treatment and both showed an anti-p190-13 CD4+ T cell response.
In conclusion novel identified p190-13 25mer peptide is able to induce in vitro a peptide-specific CD4+ T cell response in imatinib treated Ph+ALL patients. In addition this peptide includes all the epitopes for which p190-specific CTLs were naturally found in the same setting of patients. Thus it appears a good candidate for developing an immune target vaccine strategy possibly synergizing with TKIs for remission maintenance.

Part of this work is reported in: Identification of a novel p190-derived breakpoint peptide suitable for peptide vaccine therapeutic approach in ph+ acute lymphoblastic leukemia patients. XI National SIES meeting. Turin, Italy, October 6-8, 2010. Platform presentation.

Oncology and Genetics Doctoral School
Medical Genetics
XXIV cycle
Disciglio Vittoria, MS
disciglio@unisi.it
Tutor F. Mari, A. Renieri

The mirror effect of deletion and duplication on 16p11.2

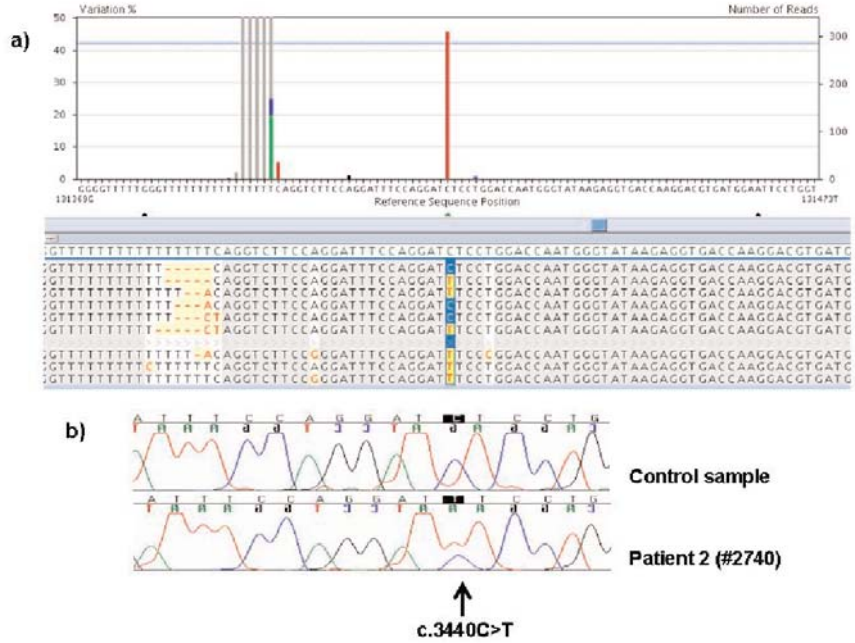
Submicroscopic 16p11.2 rearrangements are associated with several neurodevelopmental disorders, including autism, mental retardation, and schizophrenia. The common 16p11.2 region (29.5-30.1Mb) includes 28 genes many of which are expressed in nervous system.
In our Medical Genetic Unit we identified five cases (three unrelated) with 16p11.2 microdeletion and one case with 16p11.2 microduplication representing respectively 0,79% and 0,15% of 632 cases. All these patients were referred to our laboratory for aCGH testing since they showed neurodevelopmental and congenital anomalies. Our patient with 16p11.2 duplication is part of 138 duplication carriers (108 unrelated carriers) collected from over 95,000 individuals clinically referred for developmental or intellectual disabilities, psychiatric disorder or recruited from population-based cohorts. This is the largest cohort of individuals with 16p11.2 duplication described so far thanks to a collaborative effort between several Medical Genetics Unit and the Unit from University of Lousanne. This study demonstrated that duplication carriers show significantly reduced postnatal weight (mean Z-score -0.6; p=4.4x10-4) and BMI (mean Z-score -0.5; p=2.0x10-3). Moreover the duplication was associated with reduced head circumference (HC) (mean Z-score -0.89; p=7.8x10-6), while deletion was associated with increased HC (mean Z-score +0.57; p=1.79x10-5). Gene expression study conducted on 27 genes mapping within the region or nearby revealed a positive correlation between gene dosage and transcript level for all genes within the rearranged region. By contrast, the proximal genes showed no significant variation and distal genes showed a significant alteration in relative expression. These data evidence for the first time that deletion and duplication on 16p11.2 region may have opposite impacts on phenotype.



Oncology and Genetics Doctoral School
Medical Genetics
XXVI cycle
Fallerini Chiara, MS
fallerini2@unisi.it
Tutor A. Renieri

Advances in Alport syndrome diagnosis using next generation sequencing.

Alport syndrome is a hereditary nephropathy often associated with sensorineural hypoacusis and ocular abnormalities. Mutations in the COL4A5 gene cause X-linked Alport syndrome. Mutations in COL4A4 and COL4A3 genes have been reported in both autosomal recessive and autosomal dominant Alport syndrome. The conventional mutation screening, performed by DHPLC and/or Sanger sequencing, is time consuming and has relatively high costs due to the absence of hot spots and to the high number of exons per gene: 51 (COL4A5), 48 (COL4A4), and 52 (COL4A3). Several months are usually necessary to complete the diagnosis, especially in cases with less informative pedigrees. To overcome these limitations, we designed a next generation sequencing protocol enabling simultaneous detection of all possible variants in the three genes. We used a method coupling selective amplification to the 454 Roche DNA-sequencing platform (GS junior). The application of this technology allowed us to identify the second mutation in two Alport syndrome patients (p.Ser1147Phe in COL4A3 and p.Arg1682Trp in COL4A4) and to reconsider the diagnosis of Alport syndrome in a third patient. This study, therefore, illustrates the successful application of next generation sequencing to mutation screening of Mendelian disorders with locus heterogeneity.



Part of this work is reported in: Artuso R et al. Advances in Alport syndrome diagnosis using next generation sequencing. Eur J Hum Genet 2012, Jan; 20(1): 50-7.
This work is funded by: Regione Toscana project (Progetto Regionale Malattie Rare) and a donation ideally dedicated to "Graziano e Marco Laurini"to AR.

Oncology and Genetics Doctoral School
Colorectal and Gastroesophageal Disease
XXV cycle
Fontani Andrea, MD
Tutor S. Civitelli

Outcome of surgical trearment of colorectal cancer in the elderly

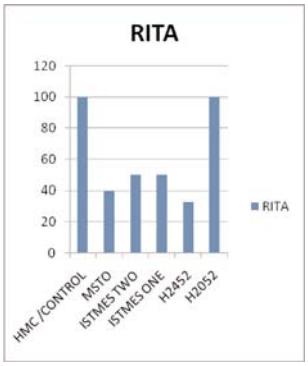
Aim of this study is to compare the clinical features and the perioperative and long term outcomes after primary surgery for colorectal cancer in the elderly population with those observed in younger patients.
In the work of the last year I studied 914 patients over the age of 55 who underwent primary surgery for colorectal cancer in our clinic from 1988 to 2008. I divided this population into two age groups: the young group (55-75 years) composed by 562 patients and the elderly group (>75 years) by 352 patients. The side of the tumor was predominantly right sided in the elderly group. The overall number of comorbidities, the emergency surgery, the overall perioperative mortality, the overall 3-years, 5-years and 10-years survival rates, the number of deaths unrelated to cancer and the number of patients lost during the follow-up, were statistical superior in the elderly group compared with the younger.
There were no statistical differences in terms of curative and palliative resections, cancer related mortality, number of stomies performed, tumor stage at presentation, sex, and post-operative hospital stay.
From the analysis of the results I have concluded that elderly patients underwent major colorectal resection have an acceptable perioperative morbidity, mortality and survival rate compared to younger patients and that age alone should not be considered a reason to deny surgery to these patients.
In this year I have collected dates of patients underwent primary surgery for colorectal cancer in our clinic from 2008 to 2010, obtaining a final data base of 978 patients over the age of 55.
Considering the improvement of surgery equipe and the progress in techniques surgery and perioperative management, I have decided to focus my study from 2000 to 2010. The study include 427 patients divided into the same age groups: The young group (55-75 years) composed by 217 patients and the elderly group (>75 years) by 210 patients. Comparing with the previous study, there were no statistical differences between the two groups in terms of: side of tumor, number of comorbidities, emergency surgery, overall perioperative mortality, number of deaths unrelated to cancer and number of patients lost during the follow-up. The improvement of surgery equipe and the progress in techniques surgery and perioperative management, reduce the morbidity and mortality of elderly patients. Elderly patients underwent major colorectal resection have a similar perioperative morbidity and mortality compared to younger patients. This study confirms that age alone should not be considered a reason to deny surgery to these patients.

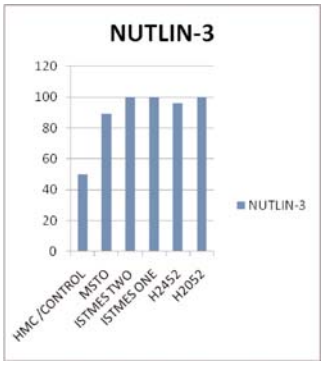
Part of this work is published in: Fontani A; et al. Outcome of surgical treatment of colorectal cancer in the elderly. Updates Surg. 2011 Dec; 63(4): 233-7.

Oncology and Genetics Doctoral School
Oncological Genetics
XXIV cycle
Forte Iris Maria, MS
irisforte@yahoo.it
Tutor A. Giordano

Pharmacological targeting of p53 effectively induces apoptosis in malignant mesothelioma cell lines

Malignant mesothelioma (MM) is a very aggressive tumor of serous membranes that is actually always fatal. The incidence, very rare until the second half of 20th century, is increasing because of a large use of asbestos in industries and in construction of buildings between the 1940 and 1979. The overall survival remains between 9 and 17 months from diagnosis. Therefore there is an urgent need to identify novel therapeutics targets. One approach to cancer therapy is based on restoration of pathways that trigger apoptosis. Reactivation of p53, the "guardian" of genome, is a new attractive strategy to inhibit cancer cell proliferation and/or induce apoptosis in tumors that have wild-type p53. This gene is mutated in approximately half human tumors. In mesothelioma p53 is rarely mutated but p53 pathway is often not functional because of inactivation of p14ARF protein that normally suppresses HDM2-dependent degradation of p53. Several approaches are currently under development to obtain molecules that disrupt p53-HDM2 interaction activating p53 tumor suppressor function. We tested in mesothelioma cell lines the activity of the Rita and Nutlin-3 molecules which were both designed to interfere with p53 and HDM2 stabilizing p53 levels although Rita directly binds to p53 whereas Nutlin-3 targets HDM2. We have chosen three mesothelioma cell lines (H2052, MSTO-211H and ISTMES-2) representing three different MM histotypes and the normal mesothelial cell line HMC-TERT (immortalized with human telomerase). These cell lines have been analysed by mRNA sequencing to found eventual p53 mutations. We next investigated the effect of Rita and Nutlin-3 on cellular growth (by MTS assay), cell cycle (by flow cytometry analysis) and apoptosis (by Annexin-V apoptotic assay). Both Rita and Nutlin-3 exhibit antiproliferative activity in all mesothelioma cell lines although Nutlin-3, differently from Rita, blocked HMC cell growth too. The effects of these two molecules on cell cycle distribution are significantly different: whereas Nutlin-3 induced a typical cell cycle arrest in G1 with reduction of S-phase percentage and a p53-dependent accumulation of p21, Rita increased S-phase population without G1 accumulation inducing p21 degradation. In addition Rita is able to strongly induce apoptosis in MSTO-211H and ISTMES-2. We propose that Rita could be a promising molecule for treatment of MM in combination with conventional therapies that are actually only palliative.



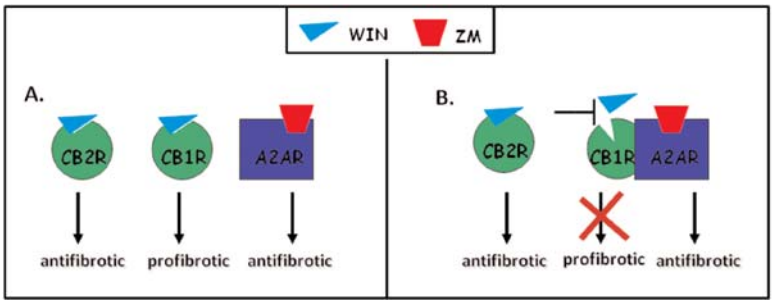


Part of this work is reported in: Indovina P. et al. New pyrazolo-[3,4-d]-pyrimidine SRC inhibitors induce apoptosis in mesothelioma cell lines through p27 nuclear stabilization. Oncogene 2011Jul 25.

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXIV cycle
Gianchecchi Elena, MS
Tutor F. Laghi Pasini

Adenosine A2A receptor activation stimulates collagen production in sclerodermic dermal fibroblasts either directly, and through a cross-talk with the cannabinoid system

Systemic sclerosis (SSc) is a connective tissue disease characterized by exaggerated collagen deposition in the skin and visceral organs.
Adenosine A2A receptor stimulation (A2Ar) promotes dermal fibrosis, while the cannabinoid system modulates fibrogenesis in-vitro and in animal models of SSc. Moreover, evidence in central nervous system suggests that A2A and cannabinoid (CB1) receptors may physically and functionally interact.
On this basis, we investigated A2Ar expression and function in modulating collagen biosynthesis from SSc dermal fibroblasts, also analyzing the cross-talk with cannabinoid receptors.
In sclerodermic cells, A2Ar expression (RT-PCR, Western blotting) was evaluated together with the effects of A2A agonists and/or antagonists on collagen biosynthesis (EIA, Western blotting). Putative physical and functional interactions between the A2A and cannabinoid receptors were respectively assessed by co-immunoprecipitation, and co-incubating the cells with the unselective cannabinoid agonist WIN55,212-2, and the selective A2A antagonist ZM-241385.
In SSc fibroblasts: (i) the A2Ar is overexpressed, and its occupancy with the selective agonist CGS-21680 increases collagen production, myofibroblast trans-differentiation, and ERK-1/2 phosphorylation; (ii) the A2Ar forms an heteromer with the cannabinoid CB1 receptor, and (iii) unselective cannabinoid receptor stimulation with a per se ineffective dose of WIN55,212-2, results in a marked anti-fibrotic effect after A2Ar blockage.
In conclusion, A2Ar stimulation induces a pro-fibrotic phenotype in SSc dermal fibroblasts, either directly, and indirectly by activating the CB1 cannabinoid receptor. These findings increase our knowledge of the pathophysiology of sclerodermic fibrosis also further suggesting a new therapeutic approach to the disease.



Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral Syndromes
XXVI cycle
Granato Felice, MD
granato@unisi.it
Tutor F. Cetta

Thymomas have an increased risk of developing additional malignancies: lack of immunologic surveillance?

The increased risk of developing an additional malignancy (AM) before or after a thymoma has not yet been fully examined. The Authors studied 68 patients affected by thymomas. Based on the WHO classification, the tumors were classified as A, AB or B (B1, B2, B3) thymomas (Fig 1). Control populations were provided by 114 patients with colorectal cancer, 108 patients with lymphoma and 123 patients with thyroid carcinoma. Patients with thymomas showed a statistically significant higher risk of developing an AM (22/68 vs 11/114 patients, 8/108, 8/123; p=0.0002). The association between thymomas and AMs was related to the thymoma histotype with B1, B2, B3 and AB thymomas showing a statistically significant higher risk of developing an AM than A thymomas (p=0.0474).
Patients affected by thymomas show a significantly higher risk of developing additional malignancies and cases that exhibit a prevalently cortical component are more closely correlated to the onset of other neoplasms. This may be related to the functions of cTECs in providing for T lymphocyte maturation through interaction with MHC complexes.

A thyroglossal duct cyst of the anterior mediastinum

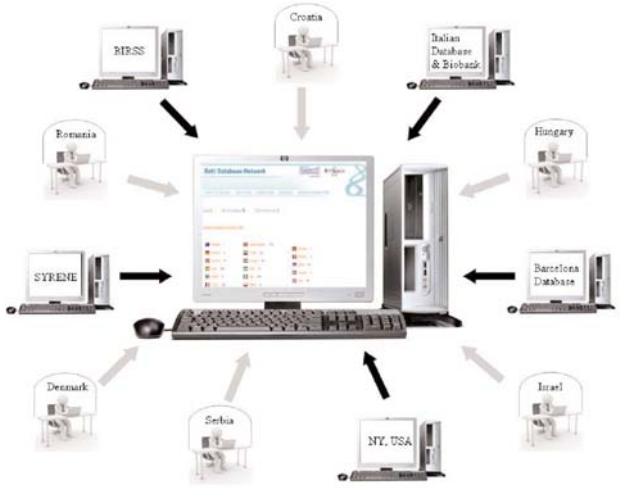
Thyroglossal duct cysts (TGDCs) are developmental anomalies arising from the embryonic thyroglossal duct. They are commonly midline cervical structures, associated with the hyoid bone. We report a 3.5cm diameter isolated TGDC of the anterior mediastinum in a 65 year old European female, treated by transcervical excision. We are aware of only one previous report of a mediastinal TGDC, and only two reports of TGDCs below the thyroid gland. Our patient was over 20 years older at presentation than the previously reported case of mediastinal TGDC. She is the first mediastinal TGDC to be reported outside Asia.
The mass was limited to the mediastinum with a normal neck on CT and clinical examination. It began below the level of the jugular notch, developing across the great vessels underneath the level of the innominate vein (Fig 2). Under general anaesthetic, a 4 cm transcervical incision made above the jugular notch. A clearly encapsuled cystic mass beneath the manubrium was easily identified. There was no fixity to surrounding structures, and it was easily excised. The post-operative course was uneventful and the early post-discharge recovery routine. Pathological examination revealed a 35x25x10mm thin walled cyst filled with mucinous material, consistent with benign TGDC.
We believe that a reasonable management strategy for mediastinal TGDC is complete excision. Local recurrence and malignant transformation are well recognised in cervical TGDCs. Excision can exclude far more common and potentially malignant causes of a mediastinal mass such as thymoma or lymphoid malignancies. TGDC should be considered as an uncommon differential diagnosis of an anterior mediastinal mass.

Part of this work is reported in: Granato F et al. A thyroglossal duct cyst of the anterior mediastinum. Ann Thorac Surg 2011 Sep;92(3):1118-20.

Oncology and Genetics Doctoral School
Medical Genetics
XXV cycle
Grillo Elisa, MS
grillo15@unisi.it
Tutor F. Ariani, A. Renieri

Rett Database Network: an integrated clinical and genetic network of Rett syndrome databases

Rett syndrome (RTT) is a neurodevelopmental disorder that covers a group of several distinctive phenotypes, namely classic Rett syndrome, the Zappella variant, the early onset seizure variant and the congenital variant. Mutations in MECP2 are found in a majority of cases but mutations in at least two additional genes, CDKL5 and FOXG1, have been shown to underlie some (usually variant) cases. Genotype-phenotype correlation studies have been performed but the results of these analyses are debated, compromising our ability to diagnose the disease and provide genetic counseling. We therefore constructed the Rett Database Network (http://www.rettdatabasenetwork.org/), using an adaptor approach to draw from preexisting databases and aiming at the worldwide collection and sharing of phenotypic and genotypic information on RTT. Through a data harmonization process, a total of 281 clinical items and 16 genetic items were generated. Among them, 60 clinical items and 7 genetic items were identified as the “minimum dataset” representing the core information. Overall, 23 clinical items contain longitudinal information. As of August 2011, this database contains information on 1840 patients with either MECP2/CDKL5/FOXG1 mutations or without mutations in known genes, coming from 11 countries. Data are entered by each center by a clinician who supervises the accuracy of data. Being a completely open network the number of patients and centers are continually growing. The Rett Database Network was constructed in order to make available to the international community data not only for the study of RTT natural history and genotype-phenotype correlation, but also to give an indication of the proportion of patients of different ages who have specific symptoms and the minimum numbers of affected individuals who have specific mutations. The network can therefore serve to identify which centers might be useful for recruiting specific groups of patients for clinical trials and for developing ‘quality of care’ measures that would be useful to drive up standards of medical management.



This work is founded by: IRSF (International Rett Syndrom Foundation) to A. R.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral syndromes
XXV cycle
Guercio Valentina, MS
guercio3@unisi.it
Tutor F. Cetta

Incidence of cancer in cantenarians and in elderly with Alzheimer's disease

The aim of my study has been to evaluate the incidence of cancer in various conditions, such as: in Alzheimer disease (AD) and among centenarians. Cancer and AD are two common disorders in old people. Recent studies showed an inverse association between AD and cancer. At the Pio Albergo Trivulzio (PAT), geriatric institute, a retroprospective study (2004-2010) was performed to detect among subjects hospitalized for AD the incidence of those with concomitant (previous or current) clinically evident malignancies.There were 79 out of 1392 (5,6%) patients (51 F, 28 M) with cancer: 25 breast cancer, 13 colon-rectum cancer, 9 prostate cancer, 5 stomach cancer, 4 liver and 4 bladder cancer. Our preliminar data confirm the existence of an inverse association between cancer and AD. Moreover, at the PAT a retrospective study (2008-2011) has been performed to evaluate among centenarians clinico- pathological characteristics, in particular the incidence of malignancies (previous or current). Twenty-eight subjects (mean age 102 y ± 2,28 range 99- 109y) were identified. Nine out of 28 (32%) had a history of malignant tumors, 3 (12%) of benign tumors. The age of diagnosis of cancer is relatively delayed in those who live to 100 y. In fact, the average age of diagnosis was 82.1 y compared to 63.2 y in the general population. Some cancers are very rare among these individuals suggesting that there are certain cancers that may be incompatible with survival to extreme old age.

Biomedicine and Immunological Sciences Doctoral School
Experimental Medicine and Atherosclerosis
XXV cycle
Kimani Jane Lucie, MD
kimani@unisi.it
Tutor A. Auteri, L. Puccetti

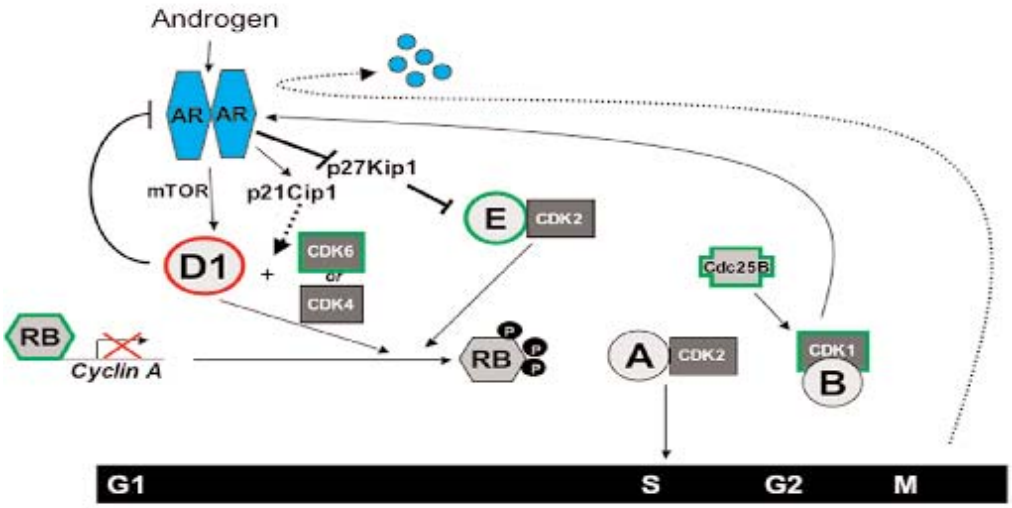
Genetic variants for the gene codificating for LOX- 1 (Lectin-like Oxidized low-density lipoprotein receptor) distribution, similarities and differences between the Caucasic population and sub saharian african population in primary and secondary prevention in relation to existing classic risk factors for atherothrombosis

LOX-1 (Lectin-like Oxidized low-density lipoprotein receptor), mediates the recognition of vascular OX-LDL receptors and makes up a key role in the pathogenesis of atherosclerosis and other cardiovascular and cerebrovascular diseases. LDL is oxidized in vascular endothelial cells to a highly injurious product that results in endothelial cell injury, which is implicated in the development of atherosclerosis. LOX-1, a lectin – like 52 –kD receptor for oxidized low – density lipoproteins (ox –LDL), is present primarily on endothelia cells. This receptor is up regulated by ox-LDL itself and by angiotensin II, by endothelin cytokines and shear stress, all participants in atherosclerosis. This receptor is up regulated in the arteries of several animal species and humans, not only on the endothelial lining, but also in the neovasculature of the atherosclerotic plaque, and is often co-localised with apoptotic cells. LOX-1 is also expressed in cells involved in atherothrombosis such as platelets and monocytes. This receptor is upregulated in hypertensive, dyslipidemic and diabetic patients. Recent studies show up regulation of LOX-1 in the ischemic-reperfused myocardium and LOX-1 inibition is associated with attenuation of atherosclerosis and associated ischemic injury. Furthermore LOX-1 has been associated with the pharmacological anti-atherothrombotic activity of several antithrombotic drugs such as statins. The present body of evidence suggests LOX-1 as a putative novel target for drug therapy. However what above described has been evidenced mainly in caucasian and asian populations whereas it seems unlikely in blacks. The aim of this study is to detect whether the 3'UTR T/C and IVS4-14 A/G polymorphisms could be associated with rising cardiovascular events ratio in a sub saharian African population with respect to classical risk factors and the putative differences with Caucasian population. As of December 1, 2011 the collection of data in the Caucasian population has been totally performed and the statistical analysis is underway. In the next month will start the evaluation of collected material for the sub saharian population and the definitive results will be available in the next six months.

Oncology and Genetics Doctoral School
Oncological Genetics
XXIII cycle
La Montagna Raffaele, MS
lamont@temple.edu
Tutor A. Renieri

Pin1 and androgen receptor in prostate cancer

Prostatic adenocarcinoma is the most frequently diagnosed malignancy and second leading of cancer death amongst man in the United States. Nonetheless, many lines of evidence indicate that the androgen receptor (AR) functions as a positive regulator of cell proliferation in Prostate Cancer, and androgen deprivation therapy is still the standard treatment for metastatic disease. AR is a member of the steroid hormone receptor subfamily of ligand-regulated nuclear receptors, and its natural ligands are testosterone and 5α-dihydrotestosterone (DHT). As with other steroid receptors, AR is a modular protein that contains an N-terminal transactivation domain, a conserved DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD). Mechanistic investigation has revealed that AR acts as a master regulator of G1-S phase progression, able to induce signals that promote G1 cyclin-dependent kinase (CDK) activity, induce phosphorylation/inactivation of the retinoblastoma tumor suppressor(RB), and thereby govern androgen-dependent proliferation. Androgen receptor (AR) interacts with β-catenin and can suppress its coactivation of T cell factor 4 (Tcf4) in prostate cancer cells. Pin1 is a peptidyl-prolyl cis/trans isomerase that stabilizes β- catenin by inhibiting its binding to the adenomatous polyposis coli gene product and subsequent glycogen synthase kinase 3β (GSK-3β)-dependent degradation. Higher Pin1 expression in primary Prostate Cancer is correlated with disease recurrence, and Pin1 expression was found markedly increased in metastatic Prostate Cancer.



Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXIV cycle
Landi Claudia, MD
landi@unisi.it
Tutor P. Rottoli

Proteomic analysis of interstitial lung diseases

Background: Sarcoidosis (Sar), Idiopathic Pulmonary Fibrosis (IPF), Langerhans cell Histiocytosis (PLCH), pulmonary fibrosis associated with Systemic sclerosis (SSc) are interstitial lung disease (ILD), all characterized by fibrosis, but with different etiopathogenesis, clinical course and prognosis. As the diagnosis of these IDL is very difficult, new diagnostic methods are strongly needed. With this purpose, proteomics of BronchoAlveolarLavage Fluid (BALF) could be a powerful method to obtain protein profiles characteristics for each diseases and for controls. These protein variation profiles could be used in association with multivariate statistical analysis to associate patients to specific pulmonary diseases.
Methods: BALF proteins obtained from 9 Sar, 7 IPF, 9 PLCH, 7 SSc, 9 smoker controls (sc) and 10 non-smoker controls (nsc) were separated by 2D-electrophoresis. The Image Master Platinum 7.0 software was used to compare the obtained electropherograms and to define characteristic differences in protein expression, that were identified by mass spectrometry. Multivariate statistical analysis, such as Principal Component Analysis (PCA), was used to associate differentially expressed protein with the different conditions analyzed.
Results: The relative volume (%V) of each interesting protein was used to perform PCA analysis. The variance in protein expression was captured by the first two principal component (PC1 21%, PC2 14,61%). Image analysis revealed distinct expression profiles of each condition and PCA demonstrated a consistent reproducibility between the biological replicates as spot maps properly segregate into six experimental groups (Sar, IPF, PLCH, SSc, sc, nsc).
Conclusion: The use of multivariate analysis allowed us to simplify the enormous quantity of proteomic data as well as to point out global trends of protein expression in BALF of patients and controls to better distinguish the different conditions. This preliminary data confirm the possibility to use 2D-elecctrophoresis and multivariate analysis as a new diagnostic method to discriminate between different interstitial lung diseases.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral Syndromes
XXIII cycle
Laviano Paolo, MD
laviano@unisi.it
Tutor F. Cetta

Study of obesity prevalence, body mass index including energy consumption measured by accelerometer and pollution related health effects in children attending primary school in Milan

The purpose of our study is to evaluate the synergy between air pollution and obesity in causing damage to health. We try to create a sort of guideline of conduct tending to modify the lifestyle. The goal is to improve health both directly by increasing the possibility of movement of children and indirectly by reducing air pollution. It's been shown that obesity, reduced physical activity and air pollution are cofactors that can cause systemic diseases.
This is most evident in children because they are more susceptible, have immature metabolism, phenomena of bio-accumulation.
Children breathe proportionately more air volume than adults so they inhale a higher concentration of pollutants, also breathe more being lower near the ground.
A campaign was carried out to the families by providing adequate information on the aims of the study. Since 2006 was undertaken a cross-sectional study about BMI in children attending primary school in the city of Milan (n= 54000). 2459 children from 9 different schools (every school representative of a district of the city) were analyzed. This sample as representative of entire population of children was obtained as a result of a statistical inference. There were 1212 (49%) girls and 1247 (51%) boys. Mean age was 8,34 +/- 1,52 years (range 6-11). From these 2459 1782 (77,2%) were within the average range of BMI. According to criteria of Cole there were 416 overweight (16,9%) whereas obese children were 144 (5,9%) (overweight + obese 22,8%). We analyzed the episodes of respiratory diseases and it was found that obese children had a higher number of episodes of asthma. At this point we have selected two schools. Daily levels of PM 10 and PM 2,5 were measured both outdoor and indoor.
Prior consent were therefore enrolled 376 children.
At first the children underwent skin prick test for allergens, analysis of fractional exhaled nitric oxide, spirometry, access hospital were evaluated and respiratory problems.
We evaluated anthropometric data and dietary habits.
It was assessed physical activity by accelerometer (Lifecorder Plus Kenz). To the children was therefore indicated the need to change the dietary habits and carry at least 1 hour of physical activity daily, enough to keep the weight in balance. At a distance of 1 year were reviewed body weight, timing, number and quantity of the meals, physical activity and rates of respiratory problems.
The data thus obtained revealed a higher percentage of accidents breathing in obese subjects compared to normal weight. There were no significant changes in weight. There was an increase in physical activity in the children and a lower percentage of asthma exacerbations despite PM concentrations were maintained throughout the year.
While accepting the fact that the samples are small and the period of observation is too limited we are convinced that this is a first step to show that pollution and obesity are capable of causing damage on susceptible individuals and changes of the lifestyle produce an increase of health.

Oncology and Genetics Doctoral School
Medical Genetics
XXV cycle
Livide Gabriella, MS
livide2@unisi.it
Tutor F. Ariani, A. Renieri

Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA

Background: Retinoblastoma is the most common primary intraocular malignancy in children. Two step inactivation of RB1 (M1-M2) represents the key event in the pathogenesis of retinoblastoma but additional genetic and epigenetic events (M3-Mn) are required for tumor development. Methods: In the present study, we employed Methylation Specific Multiplex Ligation Probe Assay to investigate methylation status and copy number changes of 25 and 39 oncosuppressor genes, respectively. This technique was applied to analyse 12 retinoblastomas (5 bilateral and 7 unilateral) and results were compared to corresponding normal retina. Results: We identified hypermethylation in seven new genes: MSH6 (50%), CD44 (42%), PAX5 (42%), GATA5 (25%), TP53 (8%), VHL (8%) and GSTP1 (8%) and we confirmed the previously reported hypermethylation of MGMT (58%) and CDKN2 (8%). These genes belong to key pathways including DNA repair, pRB and p53 signalling, transcriptional regulation, protein degradation, cell-cell interaction, cellular adhesion and migration. In the same group of retinoblastomas a total of 29 copy number changes (19 duplications and 10 deletions) have been identified. Interestingly, we found deletions of the following oncosuppressor genes that might contribute to drive retinoblastoma tumorigenesis: TP53, CDH13, GATA5, CHFR, TP73 and IGSF4. Conclusions: The present data highlight the importance of epigenetic changes in RB and indicate seven oncosuppressors never reported before in the pathogenesis of retinoblastoma. Copy number changes have been found in almost all samples but array-CGH demonstrated that they often belong to larger genomic rearrangements so that it is difficult to identify genes that effectively drive tumorigenesis. Finally, copy number changes have been identified more frequently in unilateral cases (p= 0.053), suggesting that other mechanisms could be involved in hereditary RB.

Oncology and Genetics Doctoral School
Medical Genetics
XXIV cycle
Lorenzi Bruno, MD
lorenzi7@unisi.it
Tutor F. Cetta

New insights in the physiology of the internal anal sphincter in patients treated with neoadjuvant therapy for rectal cancer: effects of chemoradiotherapy, role of the MRI and properties of the interstitial cells of Cajal

Chemoradiotherapy has been shown to reduce the local recurrence rate and is currently recommended in the treatment of patients with high risk rectal cancer. Several reports have suggested that chemoradiotherapy adversely affects anorectal function. We investigated the functional changes of the internal anal sphincter (IAS) after chemoradiotherapy. We collected IAS strips from patients undergoing abdominoperineal resection or proctectomy, mounted in organ baths and monitored the responses to electrical field stimulation (EFS) and different drugs. Five patients were treated by surgery alone, and six received pre-operative radiotherapy. We observed significant differences in the responses of control and irradiated strips to EFS ($p<0.01$), Nω-nitro-L-arginine ($p<0.01$), carbachol ($p<0.05$) and phenylephrine ($p<0.05$); and we concluded that chemoradiotherapy worsens anal continence mainly by impairing IAS function, and intrinsic nerves seem to be more susceptible to muscle cells. We then evaluated the role of the magnetic resonance imaging (MRI) in assessing the morphological changes of the IAS after chemoradiotherapy, and the possibility of correlating imaging findings with functional changes. Twelve patients were enrolled in the study and underwent MRI of the pelvis and endo-anal manometry before and after chemoradiotherapy for rectal cancer. MRI of patients receiving chemoradiotherapy showed significantly increased thickening of the IAS, likely to be related to inflammatory oedema, and alterated blood perfusion to the sphincter. These findings clearly indicated inflammatory changes of the IAS following chemoradiotherapy. The correlation with functional results is under investigation.
Specialised pacemaker cells, the interstitial cells of Cajal (ICC), expressing the proto-oncogene c-kit, has been shown to regulate the spontaneous activity of the smooth muscles in the gastrointestinal tract. Recently, ICC have been described in human IAS; however, their role in this specialised tissue is still unknown. We examined the effects of the c-kit tyrosine kinase inhibitor Imatinib Mesylate (Glivec®) on IAS strips in order to investigate the function of the ICC in the IAS. The application of Glivec® at concentration higher than 5 x 10-6 M significantly reduced the tone and the spontaneous activity of the strips. No statistical significant differences were observed after application of Glivec® in the responses to EFS. The responses to carbachol (10 μM) and phenylephrine (10 μM) were also not affected by the application of Glivec®. The identification of ICC in the IAS provides a foundation for new approaches to preclinical and clinical research. Our preliminary results suggest the ICC play a role in modulating the tone and the spontaneous activity of the IAS. Moreover, these cells may represent a target for drugs inhibiting the c-kit receptor and may provide a new approach for treating anorectal diseases.

Biomedicine and Immunological Sciences Doctoral School
Experimental Rheumatology
XXIV cycle
Maggio Roberta, MD
maggioroberta@libero.it
Tutor M. Galeazzi

Are ochronotic synoviocytes and chondrocytes able to produce ochronotic pigment?

Alkaptonuria (AKU) is a rare hereditary disorder that consists of autosomal recessive deficit of homogentisic acid oxidase (HGO) resulting in an impaired tyrosine metabolism, primarily located in the liver. This alteration leads to an increase in plasmatic levels of homogentisic acid (HGA) that gives rise to deposition of HGA melanin-like derivatives in connective tissues.
Musculoskeletal involvement is the most serious complication, leading to a severe and sometimes crippling form of arthropathy. However the mechanism leading to destructive arthropathy is still unclear.
This project focuses on the investigation to test whether synoviocytes and chondrocytes are able themselves to produce HGA, leading to ochronotic pigment.
The cultures of chondrocytes and synoviocytes were obtained from cartilage and synovium specimens from hip joint replacement surgery of 2 patients with Ochronotic arthropathy and 2 patients with Osteoarthrits as control.
HGA was quantified in the supernatants of cell cultures by high performance liquid chromatography (HPLC); HGO mRNA expression was assessed by real-time PCR techniques. Our results showed
the absence of HGO mRNA in ochronotic and control cells. Consistently HGA was not detected in culture supernatants.
Our study suggests that chondrocytes and synoviocytes are not able to neo-synthesize HGA and consequently melanin-like pigment. This observation supports the notion that they are innocent bystanders of the metabolic impairment of the tyrosine metabolism pathway.

Part of this work is reported in:
Are ochronotic synoviocytes and chondrocytes able to produce ochronotic pigment? Annual European Congress of Rheumatology, Rome, Italy - June, 2010. Poster

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXIV cycle
Mandalà Marco, MD
marcomand@hotmail.com
Tutor D. Nuti

From the endolymphatic sac to the cochlea: a novel route for drug delivery to the inner ear

Emerging therapies such as stem cells, gene therapy, and neurotrophic factors may open up new prospects for the treatment of inner ear disorders. In particular, patients with sudden hearing loss, immunomediated disorders, and Ménière's disease (MD) could benefit from these new therapies. Most of these diseases constitute a major challenge because there is no definitive and effective treatment for hearing loss.
The blood-labyrinthine barrier acts similarly to the blood-brain barrier by limiting the diffusion of systemic drugs to the inner ear. The results of local procedures (intratympanic and intracochlear delivery) are often unpredictable, and part of this variability of results, ranging from complete recovery to no improvement, may be attributable to inadequate distribution into the endolymphatic compartments.
The potentials of direct endolymphatic injections for the treatment of inner ear disorders motivated the present study aimed at verifying whether injection of drugs into the endolymphatic sac (ES) can indeed reach the cochlea and in particular the endolymph of the scala media in humans.
Patients with Ménière's disease (MD) who were candidates for ES decompression surgery were selected. To objectively define whether substances administered into the ES could reach the cochlea, we injected into the ES of these patients a mixture of dexamethasone and gadolinium.
The present study yielded both radiologic (MRI, Figure 1) and electrophysiologic (ECoG) evidence that substances injected into the human ES can reach the cochlea, presumably the endolymphatic compartment of the scala media.Audiologic and neuro-otologic follow-up, demonstrated the ES injection procedure to be safe for residual hearing and vestibular function.
The ES approach has the advantage of directly reaching the endolymphatic space, which is an ideal anatomical compartment for therapies because of its peculiarities: small size and relative isolation.
In conclusion, the ES injection procedure offers a great opportunity of reaching the cochlea and presumably the endolymphatic space safely. This novel route might constitute an advance in therapy for inner ear disorders.

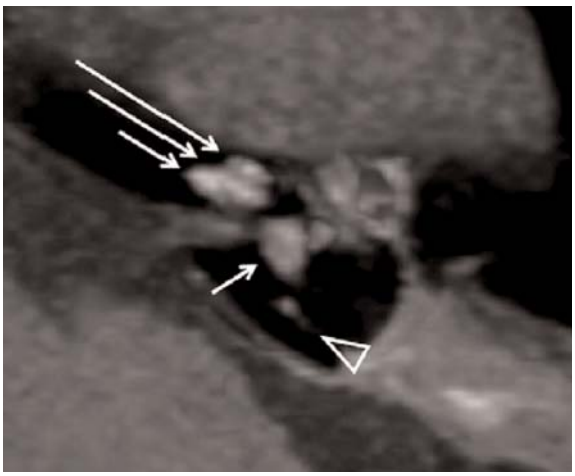
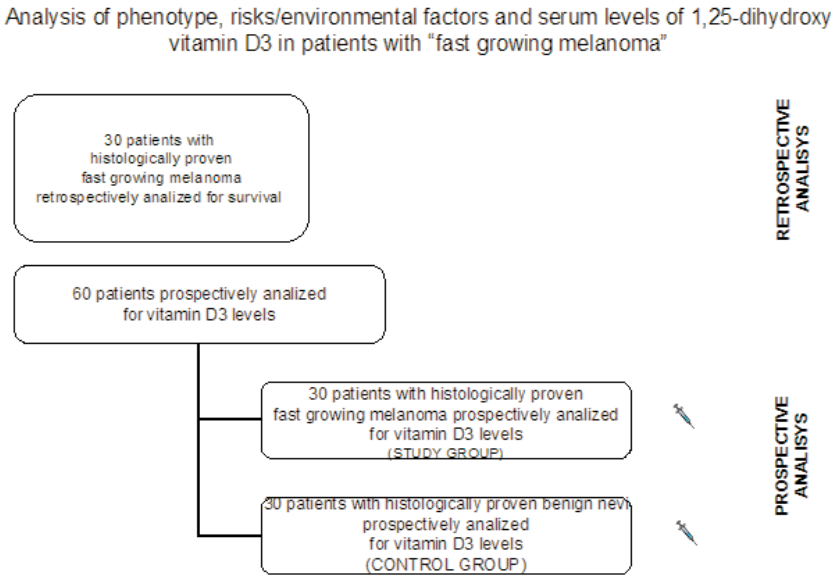


Figure 1. MRI T1-weighted spin echo, axial view images of ES GD-DEX administration (left ear). At 96 hours after ES administration: high GD signal in the vestibule (short arrow), posterior semicircular canal (arrowhead), and all the cochlear turns (3 arrows).

Biomedicine and Immunological Sciences Doctoral School
Clinical and Experimental Allergology and Immunology
XXVI cycle
Mandato Filomena, MS
Filo24k@yahoo.it
Tutor M. Fimiani

Analysis of phenotype risks/environmental factors and serum levels of 1,25-dihydroxy vitamin D3 in patients with "fast growing melanoma"

Various authors have investigated epidemiological aspects of melanoma, correlating incidence with the thickness of excised primary lesions. These studies demonstrate that the great increase in incidence of melanoma encountered in the last 20 years is largely linked to an increase in the diagnosis of medium and thin (< 2 mm thick) melanomas. This finding is not matched by a proportional reduction in thick melanomas (>2 mm thick), as would be expected. A fast-growing subtype has been identified and seems to have a role in the stabilization of total number of thick melanomas and mortality rates due to this tumour.
Evidence in favour of a role of 1,25-hydroxy Vitamin D (1,25-(OH)2D3) in the prevention of melanoma is extensive and includes epidemiological studies in vitro and in vivo. The influence of serum 1,25-(OH)2D3 on the incidence and outcome of melanoma is unknown. A recent study described reduced serum 1,25-(OH)2D3 levels in stage IV melanoma patients.
Our project proposes to analyze prospectively and retrospectively a group of about 60 patients with histologically proven cutaneous fast growing melanomas having a thickness > 2 mm or with more than one mitosis per mm2. The control groups will consist of an equal number of patients coming to our dermosurgery unit in the same period for removal of benign pigmented lesions. In these patients, we shall evaluate the phenotypic and environmental characteristics and serum levels of 1,25-(OH)2D3 for statistical correlations.



Biomedicine and Immunological Sciences Doctoral School
Experimental Medicine and Atherosclerosis
XXVI cycle
Marcelli Eleonora, XX
marcelli9@unisi.it
Tutor P. Tanganelli

Targeting the checkpoint kinase WEE1 for cancer therapy

During the past decades, extensive research has been dedicated to the development of optimal G2/M checkpoint inhibitors able to increase sensitivity to several anticancer agents. These G2/M checkpoint abrogators induce cyclin-dependent kinase 1 (CDK1) activation, thus forcing cells to enter mitosis and undergo apoptosis. Among these molecules, WEE1 inhibitors proved to be efficient in enhancing the anti-tumor efficacy of various cancer therapeutics, without affecting non-cancerous cells.
This study aims at investigating the effects of WEE1 inhibitors in combination with several anti-cancer drugs on a panel of cell lines from different tumors, such as Burkitt lymphoma (BL), mesothelioma and medulloblastoma.
BL is a highly aggressive B cell neoplasm. Despite intensive polychemotherapy regimens have proven very effective, they are associated with significant toxicities. Therefore, more rationale therapies that selectively target the molecular abnormalities of BL are needed. Recent data suggest that SRC could represent an important therapeutic target for BL. We previously tested new pyrazolo[3,4-d]pyrimidine SRC inhibitors in two BL cell lines and found that these molecules induced apoptosis and G2/M arrest through a possible new mechanism whereby SRC inhibition hinders an AKT–WEE1–CDK1 axis leading to inhibition of CDK1. Therefore, in this study, we evaluated the ability of a WEE1 inhibitor (WEE1 inhibitor II, Calbiochem) to increase the effectiveness of our SRC inhibitors, by favoring apoptosis with respect to G2/M growth arrest. We treated BL cells with the SRC inhibitors alone or in combination with the WEE1 inhibitor and we evaluated cell viability by MTS assay. We observed that the WEE1 inhibitor enhanced the SRC inhibitor cytotoxic effects in a synergistic manner. Moreover, we found that the SRC inhibitors, when used in combination with the WEE1 inhibitor, achieved an efficacy comparable to that of two chemotherapeutic agents currently used in BL therapy. We also demonstrated, by FACS analysis, that the synergy between the SRC and WEE1 inhibitors was due to a more marked induction of apoptosis and a reduced G2/M arrest compared to treatment with the SRC inhibitors alone. We also observed, by western blotting, that the WEE1 inhibitor reduced the SRC inhibitor-induced G2/M arrest by restoring CDK1 function and thus enabling the cells to enter mitosis and undergo apoptosis.
In conclusion our results suggest that SRC and WEE inhibitors could be novel attractive candidates for the treatment of BL.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral Syndromes
XXVI cycle
Martellucci Jacopo, MD
martellucci3@unisi.it
Tutor F. Cetta

Accuracy of transrectal ultrasound after preoperative radiochemotherapy compared to computed tomography and magnetic resonance in locally advanced rectal cancer

Aim of the present study was to compare the restaging results obtained by TransRectal UltraSound (TRUS), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) performed after preoperative chemoradiation with pathologic staging of the operative specimen. Methods: From January 2008 to December 2009, all the consecutive patients with locally advanced rectal cancer that underwent neoadjuvant therapy at our department were evaluated. The results of diagnostic examinations and the definitive pathological examination were considered and compared. Results: Thirty seven patients were included in the study (27 males, 73%), mean age was 65,5 years (range 45-82 years). In all patients TRUS and CT and in 20 patients MRI were performed before and after the treatment. Concerning the depth of invasion after treatment TRUS agreed with histopathology in 25/37 patients (67.5%), CT agreed in 22/37 cases (59.5%) and MRI in 12/20 cases (60%). Considering only neoplasia with stage T3, TRUS agreed in 23/24 cases (96%), CT in 19 cases (79%) and MRI in 10/12 cases (83.5%). Considering the tumors that did not exceed the rectal wall (T0,T1,T2), TRUS agreed with histology in 2/13 cases (15,5%), CT in 3/13 cases (23%) and MRI 2/8 cases (25%). Concerning the presence of positive lymph nodes TRUS agreed with histology in 28/37 cases (75.5%), while CT agreed in 21/37 cases (56,5%) and MRI in 11/20 cases (55%). The concordance between the techniques was found to be low. Conclusions: Transrectal ultrasonography resulted the most accurate method to determine neoplastic wall infiltration and lymph node involvement even after radiochemotherapy. In most cases, considering the poor correlation between the diagnostic procedures and the disagreement of the results, a restaging performed only with TRUS could be proposed, limiting the use of the other imaging methods to selected cases.

	All T stages	T3	T0,T1,T2	N
TRUS	67.5% (25/37)	96% (23/24)	15,5% (2/13)	75.5% (28/37)
CT	59.5% (22/37)	79% (19/24)	23% (3/13)	56,5% (21/37)
MRI	50% (10/20)	83,5% (10/12)	25% (2/8)	55% (11/20)

Part of this work is reported in:
- Restaging after preoperative radiochemotherapy in locally advanced rectal cancer. EAES (European Association Endoscopic Surgery), 19th international congress. Torino, 15-18 June 2011
- Accuracy of transrectal ultrasound after preoperative radiochemotherapy compared to computed tomography in locally advanced rectal cancer. Atti IX Congresso SICU, 16-18 giugno 2011, Siena

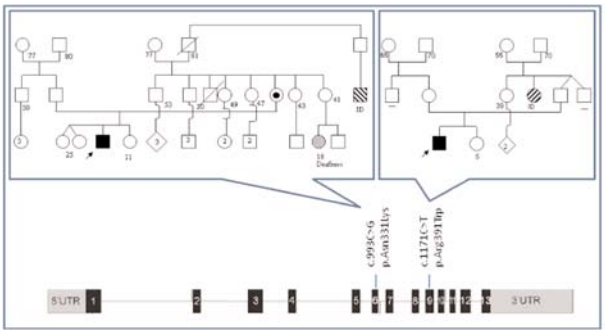
Oncology and Genetics Doctoral School
Medical Genetics
XXVI cycle
Mencarelli Maria Antonietta, MD
mariaantonietta.mencarelli@unisi.it
Tutor A. Renieri

Creatine Transporter Defect in Males With Intellectual Disability

Creatine deficiency syndrome due to mutations in X-linked SLC6A8 gene results in nonspecific intellectual disability (ID). Recently has been showed that SC6A8 gene mutations can be found in 0.8-2.2% of males with intellectual disability of unknown etiology [Newmeyer et al 2005; Clark et al 2006]. Diagnosis cannot be established on clinical grounds and is often based on the assessment of brain creatine levels by magnetic resonance spectroscopy (MRS). Considering high costs of MRS and necessity of sedation, this technique cannot be used as a first level-screening test. Likewise, gene test analysis is time consuming and not easily accessible to all laboratories.

We investigated a cohort of 258 males with ID. Among these, 231 were sporadic cases, while 27 had at least one affected brother. All patients were evaluated by a clinical geneticist and clinically recognizable syndromes were excluded. In all cases molecular analysis of FMR1 gene resulted normal. We applied a biochemical screening test, by proton NMR spectroscopy measuring urine samples. In three patients we found a consistently abnormal Cr/Crn urine ratio. A confirmatory second urine test was positive in two patients and diagnosis was further confirmed by a decreased brain creatine level and by SLC6A8 gene analysis. The first one presented a de novo mutation, while the other patient inherited a novel mutation from the mother who also has mild ID. A repeat urine test was negative in the third patient and accordingly creatine level in the brain and SLC6A8 gene analysis gave a normal result.

Our work demonstrates that urine NMR spectroscopy can be used to identify patients with a creatine transporter defect, as a rapid and useful first level screening test preceding molecular analysis. Moreover, these results presented here and those previously published by Clark stress that a significant number of males with ID may have an SLC6A8 mutation. In addition, an a posteriori re-evaluation of the clinical phenotype of positive patients confirmed that the clinical phenotype is not specific. Both patients in whom we detected an SLC6A8 mutation presented moderate ID, mostly related to speech and language, hyperactivity, behavioral disturbances, and no dysmorphic features.



Schematic representation of the two mutations identified in the SLC6A8 gene in our patients. In the upper part of the figure genealogic tree of the two families is reported.

Part of this work is published in: Mencarelli M.A. et al. Creatine Transporter Defect Diagnosed by ProtonNMR Spectroscopy in Males With Intellectual Disability. Am J Med Genet A. 2011 Oct; 155A(10): 2446-52.
This work is funded by: Telethon grants GTB07001C and GP06170 to AR.

Biomedicine and Immunological Sciences Doctoral School Sezione Osteo-Metabolic diseases
XXV cycle
Merlotti Daniela, MD
merlotti4@unisi.it
Tutor R. Nuti

Genetics and farmacogenetics of Paget's disease of bone

Paget's disease of bone (PDB) is a chronic skeletal disorder and bisphosphonates (BPs) are the treatment of choice. Mutations in SQSTM1 gene have been identified in PDB cases. We analyzed SQSTM1 mutations in 90 PDB patients treated with BPs iv. Patients were assigned to pamidronate (PAM) or zoledronate (ZOL). After 6 months, non-responders patients to PAM were crossed over to ZOL or neridronate (NER). SQSTM1 gene analysis revealed 4 different mutations in 18 patients. Patients with SQSTMI mutation showed an increased severity of disease. Therapeutic response to ZOL was achieved in 97% of patients at 12 months. Among non-responders to PAM, 93% in the NER group and 94% in the ZOL group achieved therapeutic response after 6 months from cross-over and the response was maintained in 82% and in 94% of patients at 12 months, respectively. All the 3 patients with SQSTMI mutation sustained clinical relapse from cross-over to NER suggesting that PDB patients with SQSTM1 mutation may require a more aggressive treatment. Moreover mutation prevalence is low in sporadic PDB patients, likely due to the presence of additional predisposition genes. We evaluated the effects of polymorphisms in TNFRSF11A gene. 2 SNPs, rs35211496 and rs1805034 were analyzed and both SNPs were associated with PDB in the overall cohort. The largest effect was found for rs1805034, with the C allele as the risk allele. Patients with the C allele also showed an increased disease severity especially those with SQSTM1 mutation. In vitro NFkB activity was assessed in HEK293 cells co-transfected with expression plasmids for TNFRSF11A (A192 or V192) and SQSTM1 (P392 or L392). Co-transfection with L392 and A192 produced a greater activation of NFkB signalling than P392 and V192 indicating that the polymorphism is functional. So TNFRSF11A SNPs may interact with SQSTM1 mutations to cause the severity of the disorder. Moreover, we recently identified SQSTM1-negative families with giant cell tumor (GCT). We performed a genome-wide scan in a family with PDB and GCT identifying 2 major regions on chr. 8 and 10, and an additional region on chr.20. Interestingly the linkage peak on chr.10 is downstream from OPTN gene, recently associated with PDB, while the peak on chr.8 is next to the TNFRSF11B gene, encoding osteoprotegerin.

Part of this work is reported in:
- Merlotti D, et al. Comparison of intravenous and intramuscular neridronate regimens for the treatment of Paget's disease of bone. J Bone Miner Res. 2011;26:512-8.
- Albagha OM, et al. Genome-wide association identifies three new susceptibility loci for Paget's disease of bone. Nat Genet 2011;43:685-9.
- Gianfrancesco F, et al. A non-synonymous TNFRS11A variation increases NFkB activity and severity of Paget's disease. J Bone Miner Res 2011 Oct 10.
- TNFRSF11A gene allelic variants are associated with Paget's disease of bone and interact with SQSTM1 mutations to cause the severity of the disorder. 1st IOF-ESCEO Pre-Clinical Symposium 2011,Valencia.
- Merlotti D, et al. Identification of susceptibility loci to giant cell tumor and Paget's disease of bone. ASBMR 2011 Annual Meeting, San Diego.

This work is funded by:
- John Haddad Award from Paget Foundation
- Telethon Grant 2011 (GGP11119)

Oncology and Genetics Doctoral School
Oncological Genetics
XXIV cycle
Mischitelli Monica, MS
Tutor A. Giordano, V.A. Pietropaolo

Prostate cancer scenario: STAT3 and Human Polyomavirus BK as possible actors

Recent studies evidenced that persistent activation of the Signal Transducer and Activator of Transcription 3 (STAT3) contribute to Prostate Cancer (PC). STAT3 is an excellent target for therapy since its mutant form is observed in tumor but not in normal cells.
Oncogenic infectious agents could also play a role in PC. The Human Polyomavirus BK (BKV) could be a candidate since it is an ureteliotropic virus, in vitro oncogenesis is proven and viral DNA was found in tumors. Large T Antigen (TAg) is the main oncoprotein but oncogenic properties could also be linked to the regulatory region (RR) that undergo to rearrangements and presents binding sites for host factors like p53 and c-myc. C-myc overexpression activates cell death in a p53-dependent manner. In rearranged RRs, p53 sites could be enhanced and the protein could be sequestered supporting cell transformation.
In this study, 30 fresh biopsies (pT3a androgen-refractory PC) were analysed. STAT3 target genes were chosen through ONCOMINE database and their expression was investigated using RetroTranscriptional Quantitive PCR (RT-QPCR). BKV DNA, TAg RNA and RR sequence were searched by QPCR, RT-QPCR and nested PCR for the RR, then, it was sequenced.
Results showed that c-myc was the most deregulated STAT3 target gene. Regarding BKV, two RR variants were found. Enhancement of p53 and c-myc sites was observed whereas TAg mRNA was always absent.
Results underline previous data and confirm that STAT3 could be a molecular marker for early detection of certain cancers. About BKV, the presence of RR variants allows to hypothesize that they could vary cell expression profile aiding immortalization. However, more studies will ascertain how to use STAT3 for cancer therapy and how BKV could operate on PC susceptibility.

Biomedicine and Immunological Sciences Doctoral School
Clinical Pharmacology
XXV cycle
Montilli Cinzia, MS
montilli@unisi.it
Tutor F. Laghi Pasini

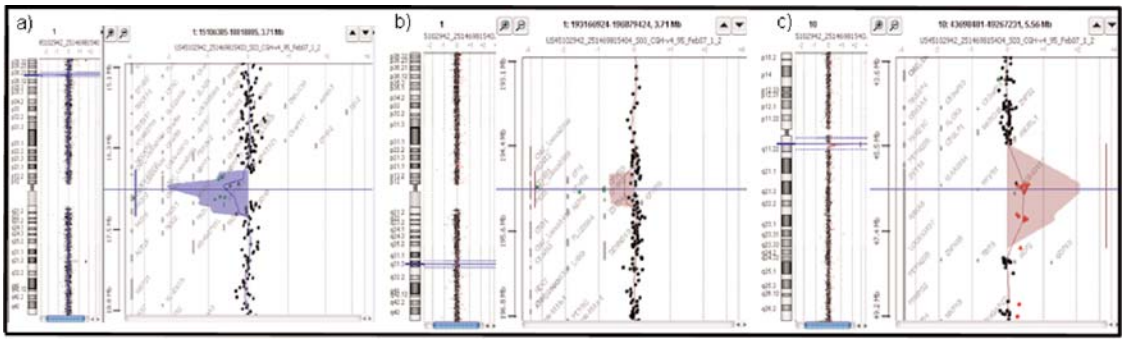
Corrected QT (QTc) interval prolongation in anti-Ro/SSA-positive patients with connective tissue disease (CTDs)

A large body of evidence links the presence of circulating anti-Ro/SSA antibodies in the mother with the risk for the newborn of developing a severe syndrome named neonatal lupus, whose main challenge is the congenital heart block.
Although being frequently detected in adult patients with autoimmune disorders, particularly CTDs, anti-Ro/SSA antibodies do not seem to be associated with the development of conduction disturbances. As a consequence, it is generally accepted that the adult heart is resistant to the antiRo/SSA– driven immunologic damage.
Recent evidence suggests that anti-Ro/SSA may be arrhythmogenic also for adults. QTc interval prolongation appears to be the most frequent electrocardiographic abnormality in adults with circulating anti-Ro/SSA. Moreover, some data demonstrated the existence of an increased risk of ventricular arrhythmias in anti-Ro/SSA–positive patients displaying QTc prolongation.
This antibody may inhibit rapid cardiac delayed rectifier potassium current through a direct interaction with the potassium ERG channel on the cardiomyocyte, thereby impairing ventricular repolarization.
We hypothesize that both the antiRo/SSA circulating levels and the number of potassium channels blocked on the cardiomyocyte membrane, and the specific anti-Ro/SSA subtype involved (52- or 60-kd subunit), may represent crucial factors to produce an interference on cardiac cell electrophysiology.
The aim of this study is to evaluate whether the anti-Ro/SSA level and specificity may represent critical factors to induce the appearance of QTc prolongation in adult patients with CTDs.
We analyzed the IgG from serum of anti-Ro/SSA positive patient by purification technique and Western blot and total anti-Ro/SSA antibodies were assessed with FEIA by the EliA system (Phadia).

Oncology and Genetics Doctoral School
Medical Genetics
XXIV cycle
Mucciolo Mafalda, MS
mucciolo8@unisi.it
Tutors F. Mari, A. Renieri

Copy Number Variations and modifier genes in Rett syndrome

Rett syndrome is an X-linked neurodevelopmental disorder predominantly affecting females and presenting with a wide spectrum of clinical phenotypes.
MECP2 mutations are responsible for two different phenotypes, classical Rett syndrome and the milder Zappella variant (Z-RTT). Previous studies demonstrated that factors such as XCI, MeCP2 mutation type and environment can influence RTT phenotype severity.
Because Copy Number Variants (CNVs) are an important source of variability in both normal and affected individuals, it could be hypothesized that the CNVs modulate the phenotypic effect of RTT syndrome. We investigated this hypothesis by comparison of array-CGH data from two discordant pairs of sisters and four additional discordant pairs of unrelated girls matched by mutation type. We also searched for potential MeCP2 targets within CNVs by ChIP-chip analysis.
We identified 29 CNVs but we did not identify one major common gene/region, suggesting that modifiers may be complex and variable between cases. However, 15 of these CNVs cold be considered “likely modifiers”. In three cases the CNV was consistent with the disease severity: i) CROCC (a potential MeCP2 target on 1p36.13), encoding a structural component of ciliary motility that is required for correct brain development, was duplicated in a Z-RTT and deleted in a classic patient; ii) CFHR1 and CFHR3, on 1q31.3, may be involved in the regulation of complement during synapse elimination and were found to be deleted in a Z-RTT but duplicated in two classic patients; iii) the duplication of 10q11.22, present in two Z-RTT patients, including GPRIN2, a regulator of neurite outgrowth and PPYR1, involved in energy homeostasis.
In conclusion, our study suggests genes for further studies in animal models or in new cellular models such as iPS. Moreover further investigation using gene expression and/or statistical analysis in a larger number of patients will be necessary to confirm these data and to define targets for future therapeutic intervention.



Part of this work is published in: Artuso R, et al. Investigation of modifier genes within copy number variations in Rett syndrome. J Hum Genet (2011), 1-8.
This work is funded by Telethon grant GTP07001C to AR.

Biomedicine and Immunological Sciences Doctoral School
Experimental Rheumatology
XXIV cycle
Muscari Isabella, MS
muscari@unisi.it
Tutor M. Galeazzi

Study of role of microRNA -223 in Rheumatoid Arthritis(RA)

Rheumatoid Arthritis (RA) is an autoimmune rheumatic disease characterized by inflammation of synovial tissue and progressive damage of the cartilage and bone tissue leading to disability. miRNAs are, indeed, tiny modulator of gene expression at post-transcriptional level and are emerging as key players in human diseases, ranging from cancer to autoimmunity. In this study we have focused our attention on miRNA expression in CD3+T-lymphocytes and biological fluids like serum and synovial fluid of RA patients.
miRNAs expression profile and subsequent Real-time PCR analysis performed on CD3+T-lymphocytes of RA patients have shown the over-expression of miR223 in comparison to control group (Psoriatic Arthritis and Systemic Lupus Erythematosus). A reduced level of miR-223 expression and, in parallel, an improvement of clinical and laboratory parameters of RA have been observed after six months of treatment with anti-TNFalpha agents.
Several studies have shown that miRNAs can be detected in a good stable form in biological fluids including plasma, serum and synovial fluids. This observation has allowed us to consider circulating miRNA as a potential useful biomarkers since they can be easily and non-invasively recovered and quantified with high sensitivity and specificity by Real time PCR. Our preliminary results have shown a dysregulation of mir223 in serum of RA patients if compared to healthy controls. suggesting that its different expression could reflect a pathological condition.
Other miRNAs like miR146 and miR155, that have been recently hypothesized to be related with RA disease, will be investigated in further studies in order to compare their expression in CD3+ T-lymphocytes, in serum as well in the synovial fluid. This study will support future utilization of miRNA as useful markers of disease activity and/or therapeutic targets.

This work is funded by:
Fondazione Italiana per la ricerca sull'Artrite (F.I.R.A.)

Oncology And Genetics Doctoral School
Oncological Genetics
XXV cycle
Olayinka Olabinjo, MS
olabinjo@unisi.it
Tutor A. Giordano

Bioinformatics approach: Data Analysis on Tumor suppressor genes in cancer initiation and progression

Tumor suppressor genes such as p53 and Rb have been discovered that when they are functioning well prevent and inhibit cancer formation but when they are inactivated, they lose their functionalities to make protein that regulate cell growth. We are interested in finding out at which point do initiation, progression and differentiation occur and what responsible for this behavior. This knowledge will be useful in production of therapeutic drugs that will help in the treatment of cancer. The challenge is to develop methods to investigate the process and determine the mechanism of their formation and to analyze their effects (negative or positive) on cells. This research focuses on data analysis with data from laboratory experiment to determine the roles of these genes in tumorgenesis. We are developing bioinformatics approach that implores the use of application such as Cytoscape and Cell designer to analyze these genes and their associated proteins while using RT PCR Array to perform data analysis. Results from Cytoscape and data analysis reveal that RB gene has been supported by its role in the negative regulation of cell cycle progression as well as the regulation of the E2F family of transcription factors. Experimental observation revealed that one of the RB genes tumor suppressor functions is its ability to promote differentiation and senescence. Further investigation on this gene and its related proteins will be conducted to determine the primary tumor suppressor of pRb pathway in cancer initiation and progression. Figure 1 below showed various relationships and interactions between RB1 with other related genes using cytoscape software.

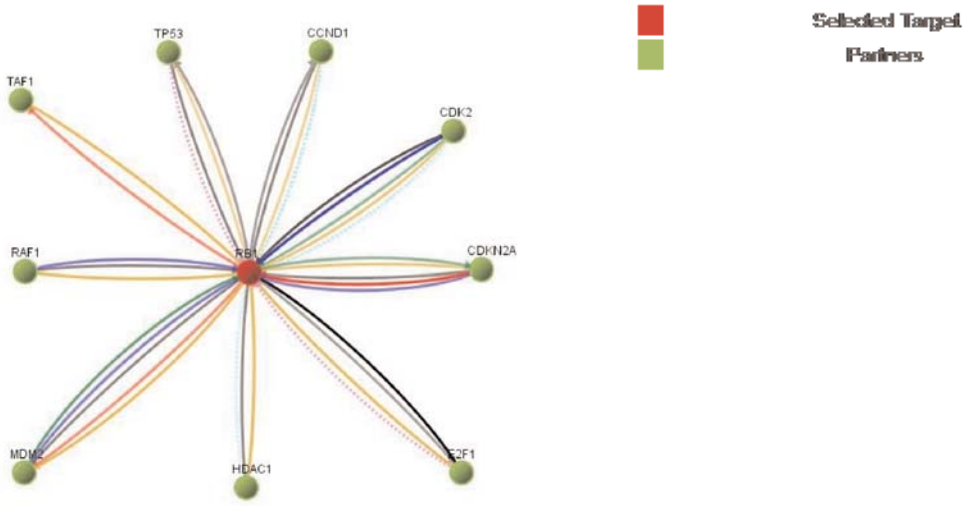
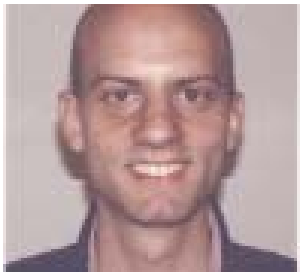


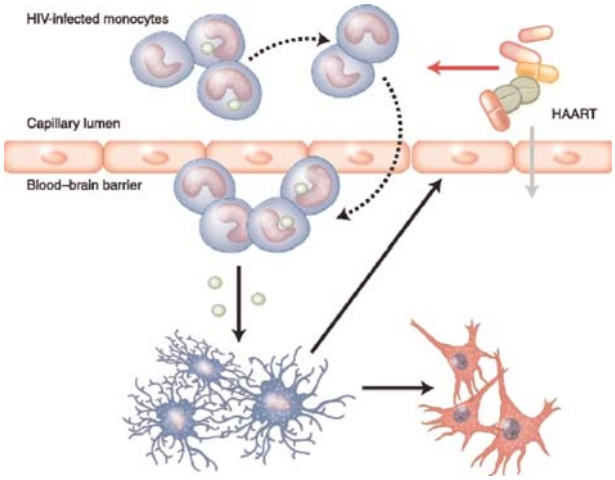
Figure 1: Diagram of the interaction of RB1 with E2F1, MDM2 and others form Cytoscape. Courtesy of SABiosciences



Oncology and Genetics Doctoral School
Oncological Genetics
XXIV cycle
Pacifici Marco, MS
pacifici6@unisi.it
Tutor A. Giordano, F. Peruzzi

MicroRNA Signature of HIV-Associated Neurological Disorders

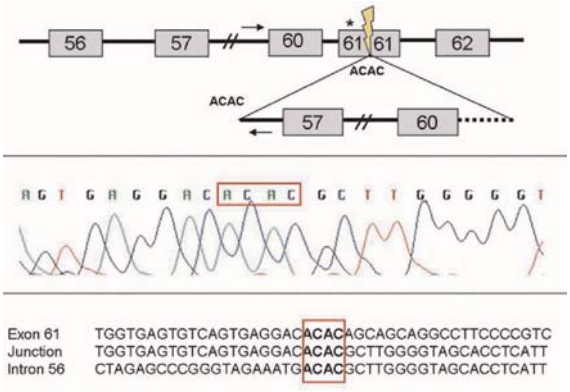
HIV-1 infection and AIDS are often associated with cognitive, behavioral, and motor dysfunctions, which severely impact the quality of life of an increasing number of HIV-infected individuals. While substantial work has been done to identify and characterize the factors released by HIV-1 infected cells and their impact on neuronal cells, accurate diagnosis of HIV-associated neurologic disorders (HAND), particularly HIV-1 encephalitis (HIVE), remains a crucial and unsolved task.
MicroRNAs (miRNAs, miRs) are small non-coding RNA species that regulate gene expression post-transcriptionally by inhibiting protein translation or promoting mRNAs degradation. Although our current understanding of the role of miRNAs in the brain pathology is still preliminary, accumulating evidence suggests the involvement of these molecules in inflammation and neurodegeneration.
Chronic stimuli as well as stress responses to ischemia, irradiation, nutrient deprivation, and oxidative stress can perturb specific miRNA expression associated with pathways involved in neurodegeneration or neuroprotection. In addition, secretion of miRNAs to body fluids, including CSF, has been associated with several illnesses, for which specific miRNA signatures can predict type of the disease and progression.
Following our previous study on the potential role of microRNAs in HIV-1 brain pathology, we hypothesize that CSF miRNA profiling of HAND, specifically HIVE, can provide us a miRNA signature unique to this pathogenesis. Therefore, our study has been focused on the identification of a miRNA signature that is a predictor of disease progression and on the role of miRNAs in the CSF as diagnostic and prognostic markers.
We obtained CSF samples from five HIV-positive patients without encephalitis and five HIVE-positive patients. Differential expression of common miRNAs was calculated across all HIVE samples to yield a quantifiable up-regulation, down-regulation, or unchanged status as compared to HIV-positive with no encephalitis. The results are indicative of a strong correlation between specific miRNA signatures in CSF and HIVE. However, analysis of a larger sample population is necessary in order to further support our hypothesis that CSF miRNAs may serve as diagnostic and/or prognostic markers.



Oncology and Genetics Doctoral School
Medical Genetics
XXIII cycle
Parri Veronica, MS
parri22@unisi.it
Tutor A. Renieri

Advances in Cohen syndrome diagnosis by MLPA and NGS

Cohen syndrome is a rare autosomal recessive disorder characterized by intellectual disability, characteristic facial features, hypotonia, pigmentary retinophaty, myopia and intermittent neutropenia. In 2003, mutations in COH1 gene (8q22) were identified as causative of Cohen syndrome. COH1 consists of 62 exons and encodes for a transmembrane protein probably involved in intracellular vesicle mediated sorting and transport of proteins. In our laboratory, traditional methods of point mutation analysis consists of denaturing high performance liquid chromatography (DHPLC) followed by automatic sequencing. The screening of a group of 96 patients with a hypotetical diagnosis of Cohen syndrome revealed 21 different point mutations, including frameshift, splicing and missense mutations. In some patients, only one or no pathogenic variant was found with traditional techniques. Since deletions have been reported as a cause of Cohen syndrome, it was possible that large rearrangements could account for missed COH1 mutations. Then, Multiple Ligation-dependent Probe Amplification (MLPA) was used to screen for COH1 large rearrangements in a group of 14 patients with a phenotype strongly suggestive of Cohen syndrome. With the use of two kits containing probes for 60/62 exons of COH1, 11 deletions and 4 duplications were disclosed. Three patients shared the same deletion spanning exons 6 to 16, that was also reported in a large Greek consanguineous family. Haplotype analysis suggested that the recurrent deletion is due to a founder effect in the Mediterranean area. Since duplications has never been reported before in Cohen syndrome patients, Long Template PCR and automatic sequencing were used to characterize one of them, spanning exon 57-60. Determination of the initial breakpoint suggested that the duplication probably lead to a frameshift and a premature truncation of the protein. Finally, since the traditional mutational analysis of the gene is expensive and time-consuming, the first experiment of Next Generation Sequencing (NGS) was set, allowing the simultaneous screening of all COH1 exons. In conclusion, incorporation of new tools as MLPA and NGS has demonstrated to increase the percentage of mutated alleles identified and to shorten the time of diagnosis. Thus, the use of these techniques is strongly suggested for Cohen syndrome molecular analysis.



Part of this work is published in: Parri V. et al. “High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome”. Eur J Hum Genet. 2010;18(10):1133-1140.

Oncology and Genetics Doctoral School
Oncological Genetics
XXVI cycle
Rizzo Valeria, MS
rizzo29@unisi.it
Tutor A.Giordano

New pyrazolo-[3,4-d]-pyrimidine SRC inhibitors induce apoptosis in mesothelioma cell lines through p27 nuclear stabilization

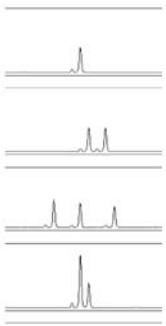
Malignant mesothelioma (MM) is a highly aggressive tumor of the serous membranes for which there is currently no specific curative modality. Recent data suggest that hyperactivation of the tyrosine kinase SRC has a key role in MM development and therefore this kinase represents an important molecular target for MM therapy. We tested new pyrazolo-[3,4-d]-pyrimidine SRC inhibitors on a panel of MM cell lines expressing the active form of SRC. These SRC inhibitors exerted a significant proapoptotic effect on MM cells without affecting the normal mesothelial cell line MET-5A, supporting a possible use of these SRC inhibitors for a safe treatment of MM. We also showed that SRC inhibitor-induced apoptosis occurred concomitantly with an increase in the nuclear stability of the cyclin-dependent kinase inhibitor p27. This finding is remarkable considering that loss of nuclear p27 expression is a well-established adverse prognostic factor in MM and p27 nuclear localization is crucial for its tumor suppressive function. To determine whether p27 stabilization has a direct role in apoptosis induced by SRC inhibition, we stably silenced the CDKN1B gene, encoding p27, in MSTO-211H and REN mesothelioma cells by transduction with lentiviral vectors expressing short hairpin RNAs against the CDKN1B transcript. Strikingly, p27 silencing was able to suppress the apoptosis induced by these SRC inhibitors in both MM cell lines, suggesting that p27 has a crucial proapoptotic role in MM cells treated with SRC inhibitors. Our findings reveal a new mechanism, dependent on p27 nuclear stabilization, by which SRC inhibition can induce apoptosis in MM cells and provide a new rationale for the use of SRC inhibitors in MM therapy.

Part of this work is reported in: Indovina P, et al. New pyrazolo[3,4-d]pyrimidine SRC inhibitors induce apoptosis in mesothelioma cell lines through p27 nuclear stabilization. Oncogene. 2011 Jul 25.

Oncology and Genetics Doctoral School
Medical Genetics
XXIII cycle
Rondinella Dalila, MS
rondinella2@unisi.it
Tutor A. Renieri

Technical improvements in prenatal diagnosis

Conventional chromosome analysis has been the gold method in prenatal genetic diagnosis for over 30 years, since the development of banding techniques in 1970. In the last years, alternative methods have been developed to detect the most common aneuploidies (trisomies 13, 18, 21, and sex chromosomes aneuploidies) in a more rapid time. QF-PCR (Quantitative Fluorescence Polymerase Chain Reaction) is presently considered the preferred technique for its cost/benefit ratio. The reporting time of this rapid test is reduced to 24-48 hours, allowing decisions on pregnancy management to be made earlier. QF-PCR has the limitation that using a restricted number of selected STRs (Short Tandem Repeat) for chromosomes 21, 18, 13, X and Y, can detect only major chromosome disorders, missing clinically significant structural chromosome abnormalities or aneuploidies on chromosomes different from those included in the assay.
Conversely, the technique of array CGH (Comparative Genomic Hybridization) is able to detect microscopic and submicroscopic chromosomal imbalances on a genome-wide scale, including all of the common aneuploidies as well as microdeletions and microduplications smaller than 100 kb in size, more below the current resolution of karyotype. Array CGH has the advantage to use direct fetal samples, deleting the need for cell culture and decreasing the overall turn around time.
In our experience of about 2 years, 1473 prenatal sample were collected (959 amniotic fluid, 514 chorionic villus) and 35 positive samples were found (3 cases performed QF-PCR, 5 cases karyotype, 27 cases both QF-PCR and karyotype). Also array CGH was performed in particular cases.
This study based on the use of QF-PCR, conventional cytogenetic analysis and array-CGH in prenatal cases suggest that QF-PCR might be used as the only approach in prenatal diagnosis for certain referral categories, replacing conventional cytogenetic analysis. Array CGH could be applied only in cases of ecographic anomalies or positive parental history. Array CGH provides diagnostic information over conventional karyotyping, therefore QF-PCR and array CGH could greatly reduce the load of conventional cytogenetic analyses in the prenatal diagnosis of chromosome disorders.



ANEUPLOIDY	N° CASES
Trisomy 13	1
Trisomy 18	7
Trisomy 21	20
Monosomy X	2
Triple X	1

POLIPLOIDY	N° CASES
Triploidy	1

OTHER REARRANGMENTS	N° CASES
Marker mosaicism	1
Structural rearrangment	1

Biomedicine and Immunological Sciences Doctoral School
Osteo-Metabolic diseases
XXIV cycle
Sebastiani Guido, MS
sebastianiguido@gmail.com
Tutor R. Nuti, F. Dotta

MicroRNA profiling during expansion and differentiation of human islet-derived precursor cells re-veals state specific microRNA signature

Generation of insulin producing cells from progenitor cells is potentially crucial to develop a proper cell therapy for type 1 diabetes. MicroRNAs are small endogenous RNAs, involved in differentia-tion, proliferation and in endocrine pancreas development. We have previously obtained in vitro human pancreatic islet derived mesenchymal (hPIDM) cells able to proliferate and to re-acquire a pancreatic endocrine phenotype forming islet-like clusters in specific culture conditions. We per-formed microRNAs profiling in hPIDM cells during expansion and their differentiation into insulin producing cells in comparison with human primary islets. hPIDM cells obtained from human pan-creatic islets, purified from 3 organ donors, were expanded in medium containing 10% FBS. For differentiation, hPIDM cells were cultured for 21 days in serum-free medium. Real-time PCR was used for gene expression analysis of 92 genes (associated to a mesenchymal or to a pancreatic en-docrine phenotype). MicroRNAs profiling (762 human miRs) was performed using TaqMan array cards and was evaluated at the following stages: human islets, hPIDM cells during expansion and after differentiation. Gene expression analysis confirmed that, during expansion, hPIDM cells ac-quired mesenchymal or stem-associated genes and lost islet-related genes. Upon differentiation, hPIDM cells re-acquired islet-associated genes with a major reduction of mesenchymal or stem-associated-genes.
MicroRNAs profiling showed a stage-specific differential expression of microRNAs involved in stemness regulation or islet function and development. Specifically, miR-302-367 cluster, involved in self-renewal and in maintenance of stemness, was absent in native islets and expressed in prolif-erating hPIDM cells, which subsequently lost its expression upon pancreatic endocrine differentia-tion. Time-course expression experiment revealed that miR-302-367 cluster was mostly downregu-lated during first 10 days of differentiation. Differently, islet-associated miR-375 was down-regulated in proliferating hPIDM and re-expressed upon pancreatic differentiation while time course experiment showed a major upregulation during first 10 days of differentiation. Interest-ingly, miR-375 expression profile in time course experiment showed a parallelism respect to the expression of insulin which was mostly upregulated during 10 days of differentiation. We also found that miR-200 family microRNAs, highly expressed in epithelial cells as well as in human is-lets cells and strongly associated with epithelial-mesenchymal transition (EMT), were strongly downregulated during expansion and re-expressed upon pancreatic endocrine differentiation. We have identified specific expression signature of stem- and mature islet-associated miRs during ex-pansion and re-differentiation of hPIDM cells, indicating that these miRs may have a crucial role in the regulation of beta-cell mass and regeneration.

Part of this work is reported in: Sebastiani G. et al. MicroRNA profiling during expansion and differentiation of human islet-derived precursor cells reveals state specific microRNA signature. EASD 2011 European Congress. 12-16 September 2011- Lisbon.

Biomedical and Immunological Sciences Doctoral School
Clinical Pharmacology
XXVI cycle
Sicuranza Anna, XX
sicuranza4@unisi.it
Tutor M. Bocchia, F. Lauria

Prognostic significance of IGHV1-69 gene in Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most common leukemia of the adult and has a heterogeneous course ranging from 2 to 20 years. Analysis of the Immunoglobulin (Ig) heavy chain (H) variable region (V) genes (IGHV) identifies 2 distinct subsets: the subset with unmutated (U) tumor IGHV (U-CLL) derives from naïve B-cells and has a severe prognosis (median survival 7-8 years), while the subset with mutated (M) IGHV derives from memory B-cells and has a good prognosis (median survival 20 years). In U-CLL, IGHV1-69 is the most tumor IG gene and is frequently rearranged to identical IGHD and IGHJ genes in the HCDR3 (complementary determining region 3). The highly similar sequences that are produced represent circa 30% of all IGHV1-69+ve CLLs and are defined as “stereotypic”. It has been hypothesized that stereotypes bind to common antigens that may regulate tumor clinical behavior. In a database of 1065 CLL patients, 250 cases carrying IGHV1-69 rearrangements were investigated for the prognostic significance of IGHV1-69 gene.
IGHV1-69 gene was UM in 220/250 cases (88%) and M in 30/250 (12%) and had “stereotyped” HCDR3 in 142/231 cases (61%) and “non-stereotyped” HCDR3 in 89/231 cases (38%).
IGHV1-69+ CLL had a significantly shorter time to first treatment (TTFT) than non IGHV1-69+ CLL. IGHV1-69+ U-CLL had a shorter TTFT than IGHV1-69+ M-CLL. TTFT was similar in IGHV1 69+ CLL with or without stereotypic HCDR3.
An exception was subset 6 with IGHV1-69/D3-16/J3 rearrangement, the only subset specific to CLL not identified in the normal B-cell repertoire and that can bind to non-muscle type II autologous myosin. Subset 6 had a very favorable TTFT and a very favorable overall survival, that were superior to the remaining stereotyped and not stereotyped IGHV1-69+ve CLL. These data suggest that prognosis is dominated by mutational status of IGH even in a homogeneous category using IGHV1-69 gene only. However, the observation, that subset 6 associates with a prognosis independent of unmutated IGH status, suggests that CLLs with CLL-specific rearrangements and that potentially interact continuously with autologous antigen(s), are anergized and result in a very indolent course with very good outcome.

Biomedicine and Immunological Sciences Doctoral School
Experimental Medicine and Atherosclerosis
XXIV cycle
Tomei Valentina, MS
tomei5@unisi.it
Tutor A. Giordano, P. Tanganelli

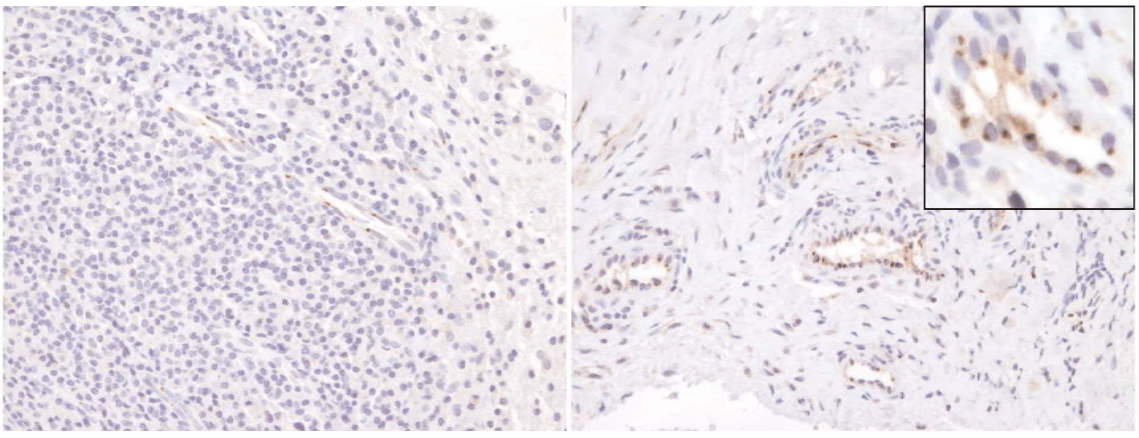
Role of Cdk9/Cyc in myogenesis

Cdk9 is a serine/threonine kinase that binds to members of the family of cyclin T (T1, T2a and T2b) and to cyclin K. It exists in two isoforms, called Cdk9-42 and Cdk9-55, because of their molecular weight. In particular, Cdk9-55 is composed by the addiction of 117 amino acids to the N-terminal domain of Cdk9-42.
This research project aims to characterize the molecular and cellular mechanisms of skeletal muscle development. The activation of Cdk9 during the formation of myotubes contributes to transcriptional activity mediated by MyoD during myogenic differentiation. In addition, the identification of Cdk9-55 and its specific pattern of expression in differentiated cells and tissues, have conducted our studies to assess the impact that this isoform may play in myogenesis. We evaluated the expression of the two isoforms during embryonic and fetal development in vivo (embryo limbs) and in vitro (primary cultures of myoblasts obtained from isolated populations of the limb bud).The results show a clear inverse correlation between the two isoforms of Cdk9. In fact, Cdk9-55 increases gradually during the process of muscle differentiation while Cdk9-42 decreases proportionally. Moreover, the significant increase of Cdk9-55 at stage E11, 5 dpc suggests a key role of this isoform in the events downstream of the muscle determination. Furthermore, the data obtained in this work have shown that increased expression of MyoD coincides with the inversion in the ratio of the two isoforms of Cdk9. To better correlate the interaction between Cdk9 isoforms and MyoD, we used mice mutant MyoD-/-. The slowdown of differentiation seen in these mice shows an alteration in the equilibrium level of the two isoforms, especially a delay in the expression of Cdk9-55. These results support previous data and attribute a major role to Cdk9-55 at the time of maximum differentiation.
All these data allow us to conclude that the expression of the two isoforms of cdk9 than being associated with a specific cell phenotype can be related to a specific function. Their timing of expression defines their likely role, in fact Cdk9-42 seems to prevail in a proliferative status and / or in the initial myogenic determination while Cdk9-55 seems to play a key role in the differentiation process.

Biomedicine and Immunological Sciences Doctoral School
Experimental Rheumatology
XXV cycle
Valcamonica Elisabetta, MS
eli.valcamonica@libero.it
Tutor M. Galeazzi, P.L. Meroni

Chemerin role in rheumatoid and psoriatic synovitis

Plasmacytoid dendritic cells (pDCs) are involved in triggering and/or in the maintenance of chronic inflammatory processes. Different studies were carried out to demonstrate the presence of pDCs in the synovial fluid and in the synovial tissue of patients suffering from Rheumatoid Arthritis (RA) an Psoriatic Arthritis (PA). The molecules involved in pDCs recruitment are not well characterized. Chemerin is the only inflammatory chemotactic factor that is directly active on human blood pDCs in vitro.
There is no evidence whether an increased expression of chemerin or of its receptors may influence the synovitis processes in RA and PA.
The aim of the study was the evaluation of: i) chemerin and its receptor expression in the synovial tissue (by immunohistochemistry); ii) chemerin levels in the synovial fluid and its mRNA expression in synovial cells from patients with inflammatory arthritis.
At present we have recruited 9 patients with RA, 1 with PA and 10 with OA (as controls).
The immunohistochemical staining showed reactivity for chemerin in all the tissue samples, however the signal was more evident in tissues obtained from patients with inflammatory synovial disease. Staining is mainly scattered around the small blood-vessels.
The evaluation of mRNA expression for chemerin receptor on the whole cell population in synovial fluids was not possible up to now. The probable reason lies in the cell damage as a consequence of the inflammatory micro-environment. New experiments on purified cell subpopulation are currently going on.
Chemerin 23 dosage in the synovial fluids displayed higher levels in samples from RA and PA patients rather than OA patients.



Expression of chemerin in synovial tissues by immunohistochemistry.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Disease and Multitumoral syndromes
XXIV cycle
Zangari Rosalia, MS
zangari4@unisi.it
Tutor F. Cetta

Mannose-binding lectin (MBL): the complement pathway activation in the pathogenesis of brain injury

Mannose-binding lectin (MBL) is a C-type lectin and it is the activator of the lectin complement pathway. Besides its important contribution to host defense against pathogens, MBL is also involved in the binding and removal of apoptotic cells leading to complement activation in an antibody and complement C1q independent manner.
Low serum levels of MBL are reported in up to 1/3 of the healthy population. MBL-deficiency arising from mutations and promoter polymorphisms in MBL2 gene has been associated with increased risk of infections and autoimmunity.
Numerous studies have acknowledged the deleterious effects of complement activation in the pathogenesis of ischemia/reperfusion injury. In this pathological condition MBL could play a crucial role due to its ability to recognize altered self-structures. Studies in murine models demonstrate that MBL deficiency or inhibition leads to diminished complement C3 deposition and neutrophil influx into the affected brain region and is associated with smaller infarct volumes and better functional outcomes. De Simoni and collaborators have demonstrated that recombinant human C1 inhibitor (rhC1-INH), an endogenous inhibitor of complement and kinin systems, is able to induce protective effect in murine models of cerebral ischemia a wide time window of efficacy. Available data obtained in the lab indicate that this neuroprotective effect is due to its direct binding to MBL and, consequently, a more efficient inhibition of complement lectin pathway.
The inhibition of this protein leads to neuroprotection and may represent a novel therapeutic target. In humans comparable data are scarce, but activation of the complement system has been confirmed in clinical studies after acute ischemic stroke.

Biomedicine and Immunological Sciences Doctoral School
Clinical Pharmacology
XXVI cycle
Zimbone Stefania, MD
stefaniazimbone@hotmail.it
Tutor: F. Laghi Pasini

P2X7 receptor and cardiac fibrosis in patients with chronic heart failure

Background. Chronic heart failure (CHF), a common cause of morbidity, hospitalization and cardiac death, represents the final result of a wide range of cardiovascular diseases, including hyperthension, coronary artery disease, valvulopathies and cardiomyopathies. In CHF, the progressive worsening of cardiac function is strictly related to a complex sequence of structural changes of the heart, collectively known as cardiac remodelling. This process, consisting in chamber dilatation, hyperthrophy and cardiac fibrosis, sees as one of the mainstay actor the cardiac fibroblast whose functional activation is deeply influenced by several cytokines, mainly TGFbeta, IL-6 and IL-1beta [1]. P2X7 receptor is a nucleotide-gated ion channel chiefly involved in the inflammatory response triggered by passive release of ATP from damaged cells. It is largely expressed in monocytes and plays a key role in promoting the release of pro-inflammatory cytokines, particularly IL-1beta and IL-6 [2,3]. Moreover, recent evidence suggests that P2X7 receptor is also expressed in fibroblasts [3] and may play a role in promoting tissue fibrosis in different body districts. However, it is not well known if such an effect is direct or indirectly induced, by enhancing inflammatory tissue damage and/or pro-fibrotic cytokine production. In an animal model of unilateral ureteral obstruction, kidneys from P2X7 knockout (KO) mice showed a reduced number of inflammatory cells (macrophages) as well as a lower immunostaining for TGF-beta and myofibroblasts when compared with wild type mice [4]. Similarly, P2X7 KO mice airway-adminestered with bleomycin to induce lung fibrosis presented dramatically reduced lung inflammation and fibrosis markers, such as lung collagen content and matrix-remodelling proteins (TIMP-1 and MMP-9) [5]. Moreover, it has been demonstrated that activation of P2X7 receptor enhanced TGF-beta1 mRNA expression in rat astrocytes [6], as well as IL-6 production in fibroblast-like synoviocytes from rheumatoid arthritis patients [3] and in dermal fibroblasts from healthy and diabetic subjects [7]. Conversely, at the moment no information exists about the expression of P2X7 receptor in human cardiac fibroblasts and its possible role in modulating cardiac fibrosis. Objectives and methods. The aim of the project is to investigate the expression and function of the P2X7 receptor in cultured human cardiac fibroblasts from patients with CHF, in comparison with cardiac fibroblasts derived from patients with cardiovascular disease but without CHF and with human cardiac fibroblast cell line as a control. The sources of human pathologic cardiac fibroblasts will be represented by (i) human right atrial (auricle) fragments obtained from patients undergoing cardiac surgical intervention with extracorporeal circulation and (ii) explanted hearts during heart transplantation. The putative expression of P2X7 receptor in these cells will be evaluated by flow cytometry and RT-PCR, while its functional role will be assessed by evaluating (i) Ca2+ fluxes (single-cell fluorescent microscopy), (ii) cytokine (IL-1-beta, IL-6, TGF-beta) expression/release (ELISA, Western blotting, RT-PCR) and (iii) collagen production (ELISA, Western blotting) after stimulation with the selective P2X7 receptor agonist 2'-3'-O-(4-benzoylbenzoyl) ATP (BzATP). Expected results. It is expected that in CHF cardiac fibroblasts P2X7 receptor is expressed and directly and/or indirectly regulates collagen production from these cells. These data may provide evidence that P2X7 receptor is actively involved in CHF cardiac remodelling, thus putatively representing a new therapeutic target for the disease.

Starting from April 13, 2006, it is possible for a PhD student to get the additional title of “Doctor Europaeus”. This title can be conferred during the final examination by the University of Siena, which is one of the Italian pioneer Universities in this field, when the following criteria are fulfilled:

- the authorization to the final PhD dissertation is accorded in the light of the reports on the thesis compiled by at least two professors belonging to two superior education institutions of two member states of the European Community different from that in which the doctorate is held;

- at least one member of the PhD dissertation board which confers the PhD qualification belongs to a superior education institution of one member state of the European Community different from that in which the doctorate is held;

- the PhD dissertation is carried out at least partially in a language of the European Community different from the national one of the state in which the doctorate is held;

- the PhD thesis must have been prepared partially following a research stay of at least three months in one member state of the European Community different from that in which the doctorate is held.

Thus, starting from April 2006 each candidate for the PhD degree could be evaluated in relation to the above reported criteria in order to decide the bestowal of qualification of Doctor Europaeus.

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

MORNING SECTION

9.00 Entering of the PhD dissertation board composed by:

- Prof. Alessandra Renieri (President)
Professor of Medical Genetics, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

- Prof. Flora Vaccarino (External Member)
Professor of Neurobiology, Yale University, USA

Thesis discussion in English language:
- "Establishment and validation of a human cellular model for CDKL5-related disorders", Mariangela Amenduni, XXIII cycle

9.45 Awarding of the PhD degree in Medical Genetics

From left to right:
Prof. Alessandra Renieri
Prof. Flora Vaccarino
Prof. Gavino Faa
Prof. Tommaso Pizzorusso
Dr. Mariangela Amenduni

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

10.00 Entering of the PhD dissertation board composed by:

- Prof. Alessandra Renieri (President)
Professor of Medical Genetics, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

- Prof. Flora Vaccarino (External Member)
Professor of Neurobiology, Yale University, USA

Thesis discussion in English language
- "FOXG1 and Rett syndrome: functional characterization and set up of an in vitro human cellular model", Roberta De Filippis, XXIII cycle

10.45 Awarding of the PhD degree in Medical Genetics

From left to right:
Prof. Flora Vaccarino
Prof. Alessandra Renieri
Prof. Tommaso Pizzorusso
Prof. Gavino Faa
Dr. Roberta De Filippis

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

11.00 Entering of the PhD dissertation board composed by:

- Prof. Alessandra Renieri (President)
Professor of Medical Genetics, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

- Prof. Flora Vaccarino (External Member)
Professor of Neurobiology, Yale University, USA

Thesis discussion in English language:
- "Advances in Cohen diagnosis by MLPA and NGS", Veronica Parri, XXIII cycle

11.45 Awarding of the PhD degree in Medical Genetics

From left to right:
Prof. Alessandra Renieri
Prof. Flora Vaccarino
Prof. Gavino Faa
Dr. Veronica Parri

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

12.00 Entering of the PhD dissertation board composed by:

- Prof. Alessandra Renieri (President)
Professor of Medical Genetics, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

Thesis discussion in English language:
- "Technical improvements in prenatal diagnosis", Dalila Rondinella, XXIII cycle

12.45 Awarding of the PhD degree in Medical Genetics

From left to right:
Prof. Tommaso Pizzorusso
Prof. Alessandra Renieri
Prof. Gavino Faa
Dr. Dalina Rondinella

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

AFTERNOON SECTION

14.00 Entering of the PhD dissertation board composed by:

- Prof. Antonio Giordano (President)
Professor of Human Pathology, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

Thesis discussion in English language
- "Androgen receptor and PIN1 in prostate cancer", La Montagna Raffaele, XXIII cycle

14.45 Awarding of the PhD degree in Oncological Genetics

From left to right:
Prof. Tommaso Pizzorusso
Dr. La Montagna Raffaele
Prof. Antonio Giordano
Prof. Gavino Faa

Thesis discussion
October 7, 2011 Centro Didattico S Maria alle Scotte

15.00 Entering of the PhD dissertation board composed by:

- Prof. Francesco Cetta (President)
Professor of Surgery, University of Siena, Italy

- Prof. Tommaso Pizzorusso (Secretary)
Professor of Neurobiology, University of Firenze, Italy

- Prof. Gavino Faa (Member)
Professor of Human Pathology, University of Cagliari, Italy

Thesis discussion in English language:
- "Study of obesity prevalence, body mass index including energy consumption measured by accelerometer and pollution related health effects in children attending primary school in Milan", Paolo Laviano, XXIII cycle

15.45 Awarding of the PhD degree in Hepatobiliopancreatic Diseases and Multitumoral Syndromes

From left to right:
Prof. Tommaso Pizzorusso
Dr. Paolo Laviano
Prof. Francesco Cetta
Prof. Gavino Faa

Thesis discussion
December 20, 2011 Centro Didattico S Maria alle Scotte

MORNING SECTION

9.30 Entering of the PhD dissertation board composed by:

- Prof. Antonio Giordano (President)
Professor of Pathology, University of Siena, Italy

- Prof. Enrico De Smaele (Secretary)
Professor of Pathology, University of Roma “La Sapienza”, Italy

- Prof. Umberto Galderisi (Member),
Professor of Molecular Biology, II University of Napoli, Italy

Thesis discussion in English language:
- “Role of Cdk9/Cyclins complex during myogenesis”, Tomei Valentina, XXIV cycle

10.30 Awarding of the PhD degree in Biomedicine and Immunological Sciences

From left to right:
Prof. Umberto Galderisi
Dr. Valentina Tomei
Prof. Alessandra Renieri
Prof. Enrico De Smaele

Thesis discussion
December 20, 2011 Centro Didattico S Maria alle Scotte

10.30 Entering of the PhD dissertation board composed by:

- Prof. Ranuccio Nuti (President)
Professor of Internal Medicine, University of Siena, Italy

- Prof. Enrico De Smaele (Secretary)
Professor of Pathology, University of Roma “La Sapienza”, Italy

- Prof. Umberto Galderisi (Member),
Professor of Molecular Biology, II University of Napoli, Italy

Thesis discussion in English language:
- “MicroRNA expression profiling reveals tissue-specific and disease-associated signatures in diabetes mellitus”, Sebastiani Guido, XXIV cycle

11.30 Awarding of the PhD degree in Biomedicine and Immunological Sciences

From left to right:
Prof. Umberto Galderisi
Dr. Guido Sebastiani
Prof. Ranuccio Nuti
Prof. Enrico De Smaele

University of Siena