

PhD Students' works

Progress Report 2009

Oncology and Genetics
Doctoral School



University of Siena



Progress Report 2009

Oncology and Genetics
Doctoral School

Molecular Biology Department

and

Human Pathology and Oncology Department

Information Engineering Department

Pediatrics, Obstetrics and Reproduction Medicine Department

Surgery Department

Surgery and Bioengineering Department

and

I.T.T. Istituto Toscano Tumori

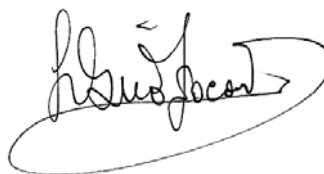
S.H.R.O. Sbarro Health Research Organization

Fiorgen Onlus

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. Silvano Focardi

A handwritten signature in black ink, appearing to read 'Silvano Focardi', enclosed within a large, loopy oval flourish.




This pamphlet was created to regroup and present together the research activities of the students of the Doctoral School in Oncology and Genetics 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 **38 PhD** students. Finally, the last pages are dedicated to the “thesis discussion days”. In the last session of thesis discussion, the qualification of “Doctor Europaeus” was conferred to two students.

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



The Doctoral School in Oncology and Genetics is constituted of 5 sections or “education trainings”:

- 1) Medical Genetics coordinated by Alessandra Renieri
- 2) Oncological Genetics coordinated by Antonio Giordano
- 3) Colorectal and Gastroesophageal Diseases coordinated by Gabriello Tanzini
- 4) Hepatobiliopancreatic Diseases and Multitumoral Syndromes coordinated by Francesco Cetta
- 5) Bioinformatics coordinated by Monica Bianchini.

In addition to the five above mentioned coordinators, the Faculty Board is composed by teachers from the University of Siena: Antonio Acquaviva, Alfio Andronico, Francesca Ariani, Alessandro Cappelli, Anton Ferdinando Carli, Maddalena Cioni, Serenella Civitelli, Paolo Frezzotti, Theodora Hadjistilianou, Marco Lorenzi, Marco Mugnaini, Francesca Mari, Giuseppe Marzocca, Clelia Daniela Anna Miracco, Roberto Ponchietti, Franco Scarselli, Maria Lucia Scampoli, Francesco Tani, Walter Testi, Paolo Toti, Luigi Verre; and by teachers from other Universities: Maurizio Genuardi from the University of Florence, Pier Paolo Pandolfi from the Cornell University, New York, Hans van Bokhoven from the University of Nijmegen, The Netherlands.

On the basis of research activity the School has signed 7 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.

The Doctoral School in Oncology and Genetics at the University of Siena trains students to carry out research in Medical Genetics and in Clinical and Molecular Oncology over a four years program. The aim of this Doctoral School is to train researchers who will be able to plan and develop competitive research proposals. The School 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 School, seminar activities, research projects, and PhD students scientific “identity card”.

The School on the basis of the high quality of the education activities and the internationalization of the scientific and teaching courses has been selected by an external board as one of the PhD Schools of the University of Siena belonging to the Graduate College Santa Chiara. The Doctoral Schools 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.

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Annual Progress Report Oncology and Genetics Doctoral School October 1, 2009 Centro Didattico S Maria alle Scotte, room 15

8.45 Welcome Addresses

Furio Pacini, Rector Delegate for International Relations of the University of Siena
Alessandra Renieri, Director of Oncology and Genetics Doctoral School

9.00 Thesis discussion (30 minutes for each one)

External Examination Board: Enza Maria Valente (Messina University), Umberto Galderisi (Napoli Second University), Gerry Melino (Leicester University), Hans Van Bokhoven (Radboud University, Nijmegen) and Michele Zappella (Versilia Hospital).

XX cycle

Causarano Vincenza (A. Renieri)

Analysis of fetal nucleic acids (DNA and RNA) in maternal plasma for non-invasive prenatal diagnosis in genetic diseases and monitoring of pregnancy complications

XXI cycle

Artuso Rosangela (A. Renieri)

Strategies for identification of new mental retardation genes (suitable for the title of "Doctor Europaeus")

Katzaki Eleni (A. Renieri)

Clinical impact of contemporary molecular cytogenetics (suitable for the title of "Doctor Europaeus")

Mancino Mario (A. Giordano)

Neuronal guidance protein Netrin-1 induces differentiation in human embryonal carcinoma cells (suitable for the title of "Doctor Europaeus")

Squillaro Tiziana (A. Renieri)

New perspectives on Rett Syndrome: the role of MECP2 gene in cellular senescence and neural differentiation

12.00 Progress report of the 4th year, XXI cycle (10 minutes for each one)

Chairmen: Gabriello Tanzini, Serenella Civitelli

Chessa Antonella (G. Tanzini)

Multiple primary malignancies: Yeta challenge

Malagnino Giuliana (F. Cetta)

Intrinsic toxicity, inflammatory potency and individual susceptibility in the occurrence of health damage from Particulate Material (PM)

Vignoli Marina (M. Genuardi)

Influence of TYMS expression and genotype on the clinical outcome of colorectal cancer patients treated with 5-fluorouracil

12.30 Progress report of the 3rd year, XXII cycle (10 minutes for each one)

Chairman: Antonio Giordano

Abbadessa Giovanni (A. Giordano)

Phase 1 dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib

postponed at 17.15 (videoconference from USA)

Benoni Simona (F. Cetta – M. Genuardi)

New cellular models for a better knowledge of the pro-inflammatory effects of environmental PM

Cisternino Filomena (F. Cetta)

Polycyclic aromatic hydrocarbons (Pahs) as a measure of pollution related health risk. Possible use as a marker of road traffic emission

Guarnaccia Valeria (A. Renieri - E. Garattini)

Characterization of the retinoid signaling pathway in breast cancer

Khadang Baharak (A. Giordano)

miRNA Expression Profiling in Malignant Mesothelioma

Marcocci Elena (A. Renieri)

Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR, one year of experience

Papa Filomena Tiziana (A. Renieri)

Array-CGH should replace traditional cytogenetic analysis for the identification of the genetic cause in miscarriages

Rizzolio Flavio (A. Giordano)

Pin1 controls cell cycle progression through interaction with pRB

Spanhol Rosseto Ariele (A. Renieri)

Rett Syndrome: mutation analysis of MECP2 in a series of 21 patients

14.00 Finger social lunch and poster viewing

Afternoon section

15.00 Progress report of the 2nd year, XXIII cycle (5 minutes for each one)

Chairman Alessandra Renieri

Amenduni Mariangela (A. Renieri)

MS-MLPA to study the contribution of epigenetic silencing in Retinoblastoma

Azzarà Annamaria (F. Cetta)

Acute Cardiovascular effects associated with air pollution: a new Pathogenetic pathway in patients with chronic obstructive pulmonary disease

Boiano Maria Giovanna (A. Giordano)

MicroRNAs are a growing class of short non-coding RNAs that negatively regulate the expression of genes

De Filippis Roberta (A. Renieri)

Molecular analysis of FOGX1 in a series of 18 Italian patients

Kola Eivana (F. Cetta)

Pollution related inflammatory and malignant diseases in subjects living in Milan, in particular, concerning the possible role of infection and airway Ph

La Montagna Raffaele (A. Giordano)

Molecular dissection of BRD4 interaction with P-TEFb complex

Laviano Paolo Angelo Maria (F. Cetta)

Air pollution from Milan affects "in vitro" sperm quality more in individuals with previous varicocele than in normal subjects or in rabbit sperm cells.

Mischitelli Monica (A. Giordano- VA. Pietropaolo)

BKV infection, inflammatory response and cancer stem cells: new actors in PC scenario

Parri Veronica (A. Renieri)

High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome

Rondinella Dalila (A. Renieri)

Identification of CDKL5 mutations in patients with the early seizure variant of Rett syndrome

16.00 pm Progress report of the 1st year, XXIV cycle (5 minutes for each one)

Chairmen Mario Chiariello, Monica Bianchini

Bruccheri Maria Grazia (A. Renieri)

Patients' collection for array-CGH analysis: identification and comparison of phenotype in two cases of deletion 16p13.1

Colecchia David (M. Chiariello)

Involvement of the Erk8 MAP kinase in autophagy

Conti Daniele (A. Giordano)

Identification of the regulatory mechanisms of Cdk2/CyclinA inhibition by pRb2/p130 protein

Crucianelli Francesca (G. Tanzini - M. Genuardi)

Methylation-specific MLPA(MS-MLPA): detection of constitutional epigenetic changes in multiple cancers

Disciglio Vittoria (A. Renieri)

RB1 mutation analysis in 23 patients affected by retinoblastoma

Forte Iris Maria (A. Giordano)

Gastric cancer and cell cycle regulation

Lorenzi Bruno (F. Cetta)

Short-term effects of chemoradiation therapy on internal anal sphincter: a human in vitro study

Mucciolo Mafalda (A. Renieri)

Side effects of de novo or inherited microdeletion/microduplications

Pacifici Marco (A. Giordano)

Function of mir-128a in normal and in pathological conditions

Sala Mariet Eliana (M. Bianchini, A. Renieri)

Project of the European Database on Rett Syndrome

Zangari Rosalia (F. Cetta)

Detection of Cell Homeostasis Imbalance in two populations subjects at different exposure to Traffic Related Air Pollution

17.00 Presentation of the PhD students program of the XXV cycle

17.15 Videoconference from USA

Abbadessa Giovanni (A. Giordano)

Phase 1 dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib

17.30 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.

Students Project Abstracts





Oncology and Genetics Doctoral School
 Oncological Genetics
 XXII cycle
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 Tutor A. Giordano

Phase 1 dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib

ARQ197 (A) is a selective, non-ATP competitive inhibitor of c-Met, a receptor tyrosine kinase implicated in tumor cell migration, invasion, and proliferation, also promoting resistance to EGFR-inhibition by driving ERBB3 (HER3)-dependent PI3K activation. Dual EGFR-Met inhibition is now proposed as a strategy for overcoming resistance to EGFR-inhibition.

Patients (pts) were enrolled in a sequential-cohort dose-escalation trial seeking to define safety, tolerability, pharmacokinetics (PK), and preliminary anti-tumor activity of A in combination with 150 mg daily oral erlotinib (E). Oral A was administered at escalating doses of 120, 240, and 360 mg bid.

25 pts (10 F/15 M; mean 60.5 yrs) received EA combination with starting A dose of 120 (8 pts), 240 (4 pts), and 360 (13 pts) mg bid. PK data reveal linear kinetics through 360 bid and no evidence of drug-drug interaction. Adverse events (AEs) considered related to combination therapy were reported in 13 (52%) of pts incl. ($\geq 10\%$ of patients) sinus bradycardia (5 pts), fatigue (5 pts), rash (4 pts), itching (3 pts), and diarrhea (3 pts). 2 pts experienced related serious AEs incl. neutropenia (360 bid) and sinus bradycardia (240 bid). 1 death on-study was unrelated to study drug. 9/10 evaluable pts demonstrated disease stabilization (SD) as their best RECIST response (5.9-27.1+ wks). Tumor regressions (2.3%-19.4%) were observed in 4/10 evaluable pts. Of note, 3/3 evaluable pts with NSCLC achieved SD for durations (14-32 wks) exceeding median PFS in BR.21 (9.7 wks).

Continuous therapy with EA combination appears well tolerated and without drug-drug interaction. While no formal MTD was identified, the dose of 360 mg bid A + 150 mg daily E is currently being investigated in a randomized trial comparing EA to E monotherapy in 2nd/3rd line NSCLC.

**ARQ 197 / Erlotinib Combination:
Biologic Profiles in 8 NSCLC Patients**

Patient ID	Prior Erlotinib	Time on Treatment (Weeks)	c-MET Amplified (by FISH)	EGFR Mutation Status	K-Ras Mutation Status
01	No	25.3	Yes	wt	wt
02	Yes	31.8	No	n/a	n/a
04	Yes	14.6	n/a	n/a	n/a
05	No	1.9	n/a	n/a	n/a
16	Yes	47+	Yes	wt	wt
21	Yes	46+	Yes	wt	wt
27	Yes	7.7	No	wt	wt
32	Yes	31+	No	wt	wt

Part of this work is reported in:

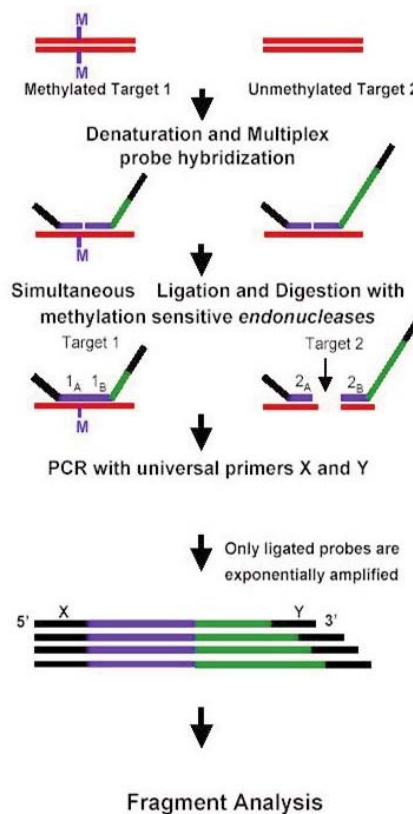
I. Laux et al. Phase I dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib. 2009 ASCO - American Society of Clinical Oncology Annual Meeting . J Clin Oncol 27:15s, 2009 (suppl; abstr 3549)
 J. Goldman et al. Phase I dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib. 2009 IASLC – International Association for the Study of Lung Cancer Annual Meeting, San Francisco, Ca



Oncology and Genetics Doctoral School
Medical Genetics
XXIII cycle
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Tutor A. Renieri

MS-MLPA to study the contribution of epigenetic silencing in Retinoblastoma

Recent studies in the field of DNA methylation have led to the awareness that epigenetic changes may represent an alternative or complementary mechanism to mutational events in tumour progression. In particular methylation of CpG islands in the promoter regions of a large number of tumour suppressor genes is observed in several human cancers. Previous studies on Retinoblastoma (RB) tissues showed frequent hypermethylation of the DNA-repair genes MGMT and MLH1 and the tumor suppressor gene RASSF1A. Methylation-specific MLPA (MS-MLPA) has been recently described as a method that allows the simultaneous identification of epigenetic changes at multiple sites. We applied this technique to study epigenetic changes in 10 RB samples and we compared results to those obtained in normal retina. Tumour tissues showed frequent hypermethylation of MGMT (70%), MSH6 (60%), CD44 (50%), PAX5 (50%) and GATA5 (30%). Since these genes are involved in DNA repair (MSH6), cellular differentiation (PAX5 and GATA5), and cell-to-cell communication (CD44), their epigenetic silencing could play an important role in RB initiation and progression. Therefore this study not only confirms the importance of MGMT inactivation, but also identifies new interesting candidate genes for RB. Aberrant methylation of these factors could play a key role in tumour development especially in bilateral cases, where chromosomal imbalances are less frequently observed.

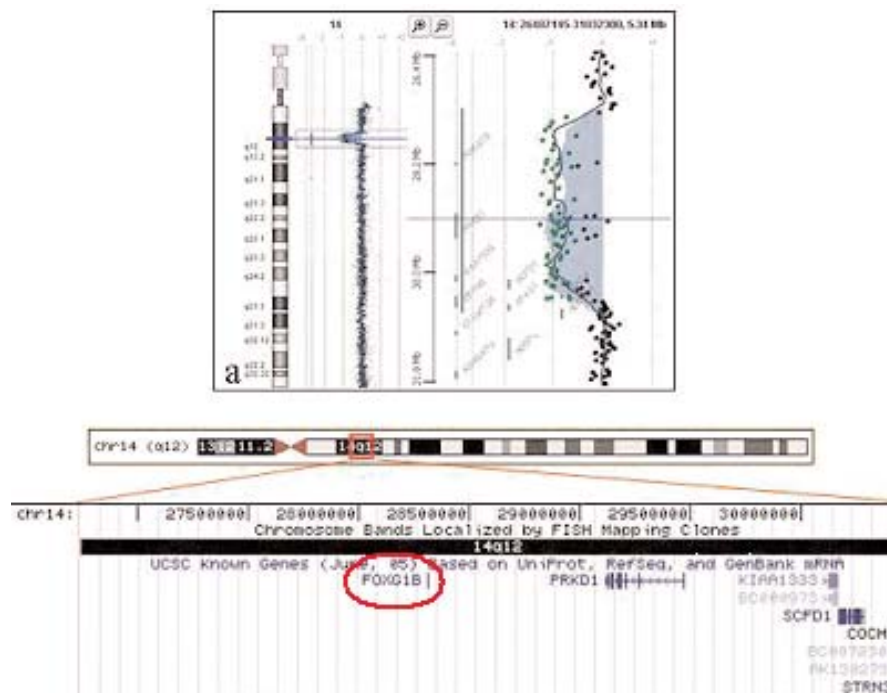


Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
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 Tutor A. Renieri



Strategies for identification of new mental retardation genes

Mental retardation (MR) affects approximately 2-3% of children and it is characterized by reduced cognitive functioning, with an intelligent quotient <70, and severe deficits in basic adaptive and social skills. MR is divided in: syndromic MR, which is accompanied by other manifestations, and nonsyndromic MR, which is characterized by reduced cognitive function only. The underlying causes of MR are extremely heterogeneous. In order to identify new causative genes we have employed two strategies, able to identify deletions and duplications with high resolution throughout the genome: linkage analysis using STR markers on X chromosome; analysis of copy number variations using SNP microarray and array CGH. The last technique identified a de novo 3 Mb deletion on chromosome 14q12 in a girl with dysmorphic features and a Rett-like clinical course. The deleted region included FOXP1. Mutation analysis in negative Rett patients identified this gene as responsible for the congenital variant of Rett. In a second patient, the SNP array revealed a 3Mb deletion on chromosome 9 and an intragenic deletion of CNTNAP2. The first deletion is not present in the three affected brothers of the patient and the intragenic deletion will be thus tested. Linkage analysis in a family with X-linked semi-dominant MR identified a candidate region in Xp11.4. Since male patients show microcephaly and mental retardation while females present isolated microcephaly, we focused our analysis on CASK which has been found mutated in patients with microcephaly.



Part of this work is published in:
 Mencarelli M, et al. Novel FOXP1 mutations associated with the congenital variant of Rett syndrome. J Med Genet. 2009 Jul 2.



Doctoral School of Oncology and Genetics
Hepatobiliopancreatic Disease and Multitumoral Syndromes
XXIII cycle
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Tutor F. Cetta

Acute Cardiovascular effects associated with air pollution: a new Pathogenetic pathway in patients with chronic obstructive pulmonary disease

Increased exposure to air pollution causes both acute and chronic inflammation that could adversely affect the cardiovascular system. A working hypothesis to explain PM related cardiovascular (CV) damage includes effects of systemic inflammation, shown by elevated C reactive protein, blood leukocytes, platelet, fibrinogen and increased plasma viscosity. Another hypothesis is direct action of particles that have become blood born, after passing through the alveolar-interstitial filter (Mills, 2009). However, in most of cases, acute severe CV effects occur within 1-2 hours after the PM pollution peak, and it is unlikely that these mechanisms could be responsible for early cardiac arrest (Cetta, 2008).

The aim of the present study has been to evaluate whether other mechanisms could be responsible or co-responsible for acute CV effects.

Methods: A cross-sectional survey was performed, comparing daily values of PM in Milan, measured by stationary monitors with the rate of hospital admission. Environment pollution data were compared with the clinical and functional status of two groups of old patients living in public hospices, close to or far from located cross to or far from main cross roads.

Results: More than 20.000 patients had hospital admission because of respiratory or CV complications during 2008. In particular, acute admissions to hospital for CV diseases during pollution peaks were more frequent in patients with previous COPD.

Conclusion: We hypothesize that another mechanism could also occur, in addition to ROS mediated systemic inflammation and autonomous system alteration via sensory nerves in the respiratory tract, in patients with COPD, chronic air tract infection or emphysema. In these patients, increased PM inhalation during pollution peaks could determine increased occurrence of endoluminal "plugs", consisting of mucus, bacteria, cellular debris and PM particles, which facilitate acute obstruction of a variable proportion of bronchiolo-alveolar ducts, in already compromised patients.

This work was supported by the Flagship Project, PROLIFE, City of Milan, Italy.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral Syndromes
XXII cycle
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Tutor F. Cetta, M. Genuardi



New cellular models for a better knowledge of the pro-inflammatory effects of environmental PM

Health effects (asthma, COPD, pulmonary fibrosis) from long-term exposure to environmental particulate matter (PM), show similarities to autoinflammatory and autoimmune rheumatic diseases.

The final clinical outcome is determined by oxidative stress, ROS generation, and activation of NF κ B pathways, increase of IL6, IL8, and TNF α .

The aim of this study has been to assess the effects of various types of PM on human synoviocytes and fibroblasts, and to evaluate the occurrence of pro-inflammatory effects. Daily levels of different PM were measured, inside and outside 2 schools and 2 hospices in Milan, and aliquots were incubated, at increasing concentrations, with human type B synoviocytes and fibroblasts.

Preliminary data showed both an increased production of IL-6, IL-8 and functional activation of P2X7R.

In particular, PM-host interaction triggered NF κ B pathway, whereas various components of PM interacted with host cells and tissue, generating a wide variety of membrane alterations.

It is suggested that occurrence and severity of evident diseases is not only related to intrinsic toxicity of various pollutants, but also to host-particle interactions and to the type of response, the entity or grade of reaction.

Our hypothesis is that PM-related diseases are determined not simply by an inflammatory mechanism, but more complex responses are generated (autoinflammatory and autoimmune diseases), occurring only in pre-disposed subjects, such as other epidemiological studies have shown.



Oncology and Genetics Doctoral School
Oncological Genetics
XXIII cycle
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Tutor A. Giordano

MicroRNAs are a growing class of short non-coding RNAs that negatively regulate the expression of genes

Mature miRs derive from longer transcripts (pri-miRNAs) which are processed to shorter hairpin precursors (pre-miRNAs) by the action of different enzymes. Different studies strongly support the idea that miRNA gene expression mechanism is based on complementary mRNA sequences inactivated by cleavage in a fashion similar to RNAi, while pairing with partially complementary sequences in the 3' UTR of target mRNAs can either repress translational efficiency or induce transcript decay. Present estimates suggest that miRNAs may regulate the expression of up to 33% of human transcriptome by few hundred of human miRNAs currently known to exist. Besides negatively regulating the expression of genes involved in proliferation, apoptosis, development, differentiation and antiviral defense, miRNAs have also a causal role in cancer. Even though miRNAs have been identified in various tumor types, showing that different sets of miRNAs are usually deregulated in different cancers, their function and role in this multifactorial disease is still unclear and/or unknown. The prediction of the targets of miRNAs is one of the key factors in facilitating the elucidation of miRNA function in cancer.

We propose to study the involvement of miRNAs in cancer by developing novel computational methods for miRNA findings and novel computational prediction of miRNA target sites. We will perform microRNA gene target analysis using both “wet” and “dry” approaches. This project might narrow our future research toward investigation of gene targets that regulate different signaling pathways and their implication in cancer pathogenesis and development.

In order to study miRNA expression profile in PC, we are using a panel of four human cell lines as an in vitro model for prostate carcinogenesis and progression. These four cell lines from ATCC (WPE1-NA22, WPE1-NB14, WPE1-NB11, and WPE1-NB26) are tumorigenic cell lines with progressive malignant characteristics derived from RWPE-1, immortalized, non-tumorigenic, human prostate epithelial cell line. We choose this approach to mimic multiple steps in progression from normal epithelium to prostatic intra-epithelial neoplasia, and then to invasive cancer. Analysis of miRNA expression profiles from this panel of human cell lines is currently under study by real-time PCR approach. Also, we are evaluating “in vitro” miRNA labeling with different novel and known approaches.

Oncology and Genetics Doctoral School
 Medical Genetics
 XXIV cycle
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 Tutor A. Renieri



Patients' collection for array-CGH analysis: identification and comparison of phenotype in two cases of deletion 16p13.1

Mental Retardation (MR) has a prevalence of ~2-3%, and are often seen in conjunction with growth retardation, dysmorphic features, and various congenital anomalies but no etiology is recognized in ~ 50% of affected individuals. During the screening of 308 patients with facial dysmorphisms, mental retardation and/or congenital anomalies we have identified a deletion, within 16p13.1 in two patients.

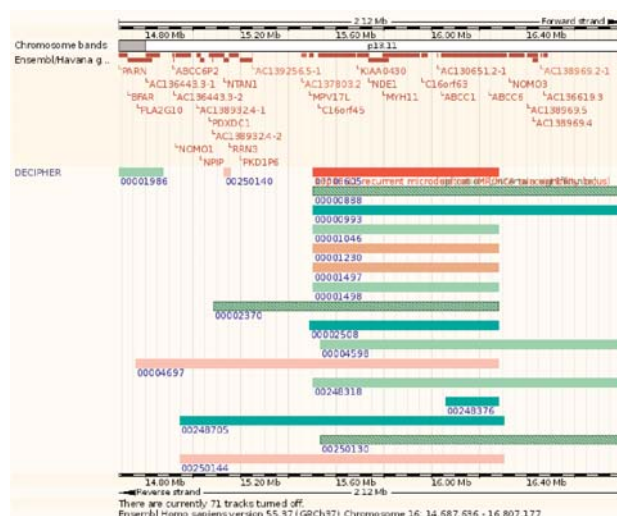
More than 10% of the euchromatic region of the p arm of chromosome 16 is rich of low copy repeats so region is frequently cause of microdeletions. Microdeletion was first observed by Hannes et al (2008) using a BAC array CGH screen of 1027 individuals with mental retardation (MR) and/or multiple congenital anomalies (MCA). All having moderate/severe MR, with epilepsy in 2 of the 3.

We compared the clinical features of our patients with 16 p13.1 deletion with other 3 rare case overlap described in the DECIPHER DATABASE. The phenotype of our 16 p13.1 deletion is characterized by MR, behavioural problems, dysmorphic features, macrocephaly and obesity.

The first patient (Patient 250140) presents a small deletion 16 p13.1p13.11 spanning about 0.15 Mb and a duplication on chromosome 2q13q13, spanning about 0.14 Mb and encompasses ~3 genes (PDXDC1, NTAN1, RRN3). The second patient (Patient 250144) has a longer deletion 16 p12.3;p13.12, spanning about 2.12 Mb and encompasses 14 genes (PLA2G10, NPIP, PDXDC1, NTAN1, RRN3, MPV17L, C16orf45, KIAA0430, NDE1, MYH11, C16orf63, ABCC1, ABCC6, NOMO1). In both cases investigation of the parents is not completed.

Deletions of 16p13.1 are significantly associated with patient phenotype, its has an incomplete penetrance as demonstrated by different phenotype compared.

So we can conclude that 16p13.11 deletion is a susceptibility locus for MR/MCA, its is in itself sufficient to cause the phenotype, but further data is needed before reaching a definitive conclusion.





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Analysis of fetal nucleic acids (DNA and RNA) in maternal plasma for non-invasive prenatal diagnosis and monitoring of pregnancy complications

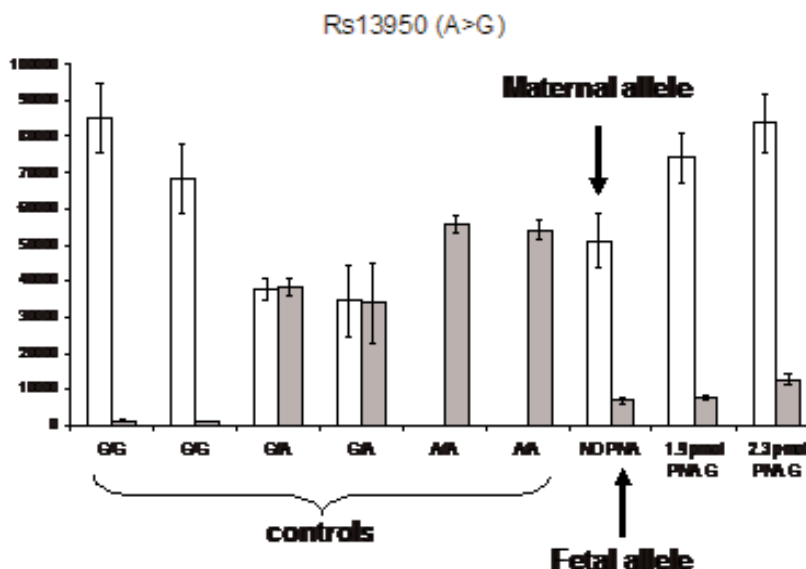
The discovery of fetal DNA/RNA in maternal plasma opened up new possibilities for non-invasive prenatal diagnosis and monitoring of pregnancy complications.

We developed a microarray strategy for direct detection of paternally inherited alleles in maternal plasma by the use of highly sensitive substrates avoiding fetal DNA enrichment. This was applied to the detection of fetal DNA polymorphisms in the cystic fibrosis conductance transmembrane regulator gene in maternal plasma. The high sensitivity of the substrate may be applied to the identification of any fetal sequence variation for non-invasive diagnosis of a variety of genetic diseases.

Preeclampsia (PE) and fetal growth restriction (FGR) are two of the most common pregnancy complications. The identification of early markers of the development of these pathologies is crucial for early therapeutic interventions, aimed at preventing maternal and fetal injuries.

Concerning the study of plasma markers, we evaluated Corticotropin-releasing hormone (CRH), long pentraxin 3 (PTX3) mRNA and protein levels in women with overt PE and FGR or at risk for these pathologies.

Due to the scarcity of RNA in plasma, we introduced substantial improvements to the real-time PCR protocol, including a new extraction method and a preamplification step. CRH mRNA and PTX3 protein levels were significantly increased in overt FGR or PE, CRH and PTX3 mRNA were significantly increased in the latest plasma sample of women who subsequently developed PE.



Part of this work has been published:

Bruno F. et al. High-sensitive microarray substrates specifically designed to improve sensitivity for the identification of fetal paternally inherited sequences in maternal plasma. *Clinical Chemistry and Laboratory Medicine*. Volume 47, Issue 7, Pages 818–823.

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Multiple primary malignancies: Yeta challenge

We analyzed databases on 815 subjects who underwent surgery for colorectal cancer from 1985 to 2005 periodically followed up by clinical and instrumental examinations.

The aim of our follow up program is the detection of the first cancer representation and the prevention of metachrone colic or extracolonic malignancies. We detected MPM (Multiple Primary Malignancies) in 120 out of 815 patients (14.72%). Metachronous malignancies are more frequent than synchronous ones (75 vs 45). The mid time between two neoplasms is 40 months.

Among metachronous neoplasms, extracolonic ones are more frequent. In subjects that developed a colorectal cancer after a first colorectal cancer, the last pancolonoscopy was performed 22.5 months before. Three metachronous tumors found six months after first surgery have been considered misunderstood synchronous malignancies. The colic neoplasia localization, proximal or distal to the right flexure, is similar in patient with unic colorectal cancer and in patient affected by MPM.

In 15 MPM patients suggestive criteria for hereditary colorectal cancer are present and these subjects have been invited to genetical counselling and we are studying results.

Skin, breast and colonrectum are in this order the most frequent sites interested by multiple primary malignancies. Colonrectum is involved by MPM in a percentage from 30 % to 50 % according to Literature.



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Polycyclic aromatic hydrocarbons (Pahs) as a measure of pollution related health risk. Possible use as a marker of road traffic emission

Interactions between air pollution and human health is complex, because of the complexity of airborne pollutants and that of host response, which greatly varies, according to individual susceptibility.

To try to distinguish the traffic related PM component as a separate entity, is noteworthy.

Polycyclic aromatic hydrocarbons (PAHs), on the basis of their mutagenicity when matched in vitro with human cells, are considered as main responsables of the mutagenic power of particulate material (PM), and classified as probable or possible carcinogens (2A and 2B).

One major outcome of the present research will be the possible use of PAHs as a risk marker of traffic related pollution. However, their possible use as a parameter of potential health damage does not imply that clinical adverse effects in individuals is determined by or related to their intrinsic mutagenicity or carcinogenicity. In fact, the pathophysiological consequences of a substance such as benzo(A)pyrene which is diluted 1:100000 in the atmosphere and is even more diluted when interferes with air tract cells are likely very little. On the contrary, there are other substances which are present in fuels, as well as in tire debris or brake linings (which are part of road emission) which could play a major role in traffic related health effects. Further studies are warranted, in particular to stress from one hand the specificity of PAHs as traffic related pollutants and, on the other hand, the risk of oversimplification, while extrapolating the actual role of PAHs in the occurrence of health damage in single individuals.

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Involvement of the Erk8 MAP kinase in autophagy

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, cell survival and apoptosis. The last identified MAPK is Extracellular signal-regulated kinase 8 (ERK8) whose activity is modulated by serum, DNA damaging agents and different activated oncogenes, in particular Src, Abl and RET/PTC3.

In order to identify proteins contributing to ERK8 biological activity, a two-hybrid screening of a mouse brain library has been performed using the C-terminal domain of ERK8 as bait. This assay allowed the identification of multiple positive clones, encoding for proteins acting in autophagic process. Several data already suggest that these proteins may be involved in the genesis of autophagosomal membranes. We, therefore, decided to focus our attention on autophagy and on the potential involvement of ERK8 activity in the control of this process.

First of all, we cloned human full-length expressed sequence tags (ESTs) cDNA of the putative interacting proteins in a human expression vector under the transcriptional control of the Elongation Factor 1 (EF-1), as a basic tool to verify interaction in co-immunoprecipitation and pull-down assays. At the same time, to assess if ERK8 affects autophagy, we set up methods to follow vesicles formation, through confocal microscopy assay, and to value autophagic flux, quantifying common autophagy marker on western blotting.

Short-term objective is to biochemically characterize the interaction between Erk8 and such positive clones and to understand the biological significance of these interactions in the context of the autophagic process. Therefore, as autophagy has been already proposed to affect cancerogenesis by multiple mechanisms, we expect that Erk8 will be able to control different aspects of cell proliferation and transformation. Understanding the machinery involved in the regulation of Erk8 activity and the biological processes in which this kinase participate to, will therefore provide us a rationale for the development of pharmacological inhibitors for Erk8 and, hopefully, for their use for prevention and therapeutic intervention.



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Identification of the regulatory mechanisms of Cdk2/CyclinA inhibition by pRb2/p130 protein

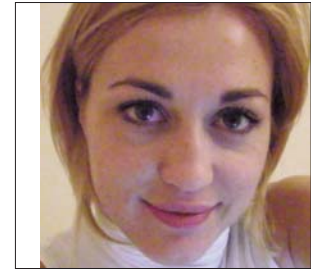
Retinoblastoma (RB) family proteins pRb, p107 and pRb2/p130 are important cellular factors which play a well-recognized role as tumor and growth suppressors. These proteins are actively involved in the negative control of the cell cycle and their function is modulated via complex homeostatic processes, most of them involving post-translational regulation of their phosphorylation status. Interestingly, the family members p107 and pRb2/p130 share the ability to physically interact and inhibit the kinase activity of the Cdk2/Cyclin A and Cdk2/Cyclin E complexes. Regarding pRb2/p130, its inhibitory effect on Cdk2/Cyclin A activity has been attributed to the "spacer" region. Recently, a 39 aa-long pRb2/p130 spacer-derived peptide (Spa310, aa 641-679) was selected as the sequence responsible for Cdk2/CyclinA inhibition. The aim of our study is to identify the minimal portion of this peptide (Spa310) that binds to and inhibit cyclin A- and cyclin E-associated kinase activity.

To investigate the protein-protein interacting regions we produced the Cdk2, CyclinA and SPACER recombinant proteins from *E. coli*. Several tests were performed in order to find the best conditions for the expression and the purification of the above mentioned proteins. Finally we were able to achieve an optimal yield of expression and a good quality purification.

The next steps will involve the use of the purified proteins in several experimental procedures: first they will be investigated by crystallography to further understand their binding structure and later on they will be used for kinase activity tests and for cytotoxicity tests on tumoral cell lines.

We believe this model to be useful for the rational development of small peptides or peptidomimetic kinase inhibitors for negative cell cycle regulation in cancer cells.

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Methylation-specific MLPA(MS-MLPA): detection of constitutional epigenetic changes in multiple cancers

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. Recently, evidence has been accrued showing that constitutional epigenetic silencing can mimic genetic loss of function mutations by abolishing gene expression.

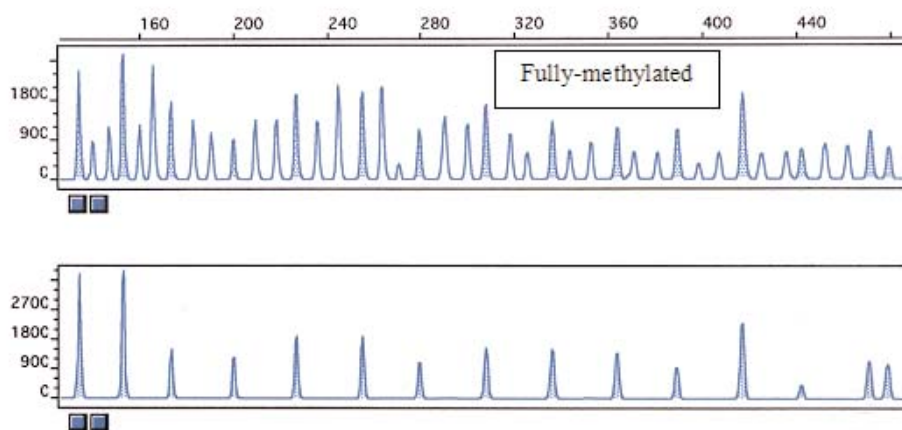
This project will be focused on the investigation of aberrant constitutional DNA methylation in mutation-negative patients who are suspected to be at high risk of cancer.

Patients will be selected based on the presence of multiple primary tumors and/or early onset tumors with or without a positive family history. The search will be performed on non cancerous tissues. In addition, we will analyze tumor tissue, when available.

Methylation analysis will be performed by a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay that allows simultaneous semiquantitative detection of CpG methylation in 26 target sequences from tumor suppressor genes.

Constitutional methylation will be confirmed by bisulphite sequencing. In addition, RNA will be analyzed, when available, to investigate allelic imbalances and quantitative expression by Real-Time PCR.

So far, DNA samples have been collected from 51 unrelated probands. The MS-MLPA technique has been set up on 2 control samples, and 4 samples from patients with multiple tumors have been investigated. No alteration of MS-MLPA peak ratios has been observed in this small subset.

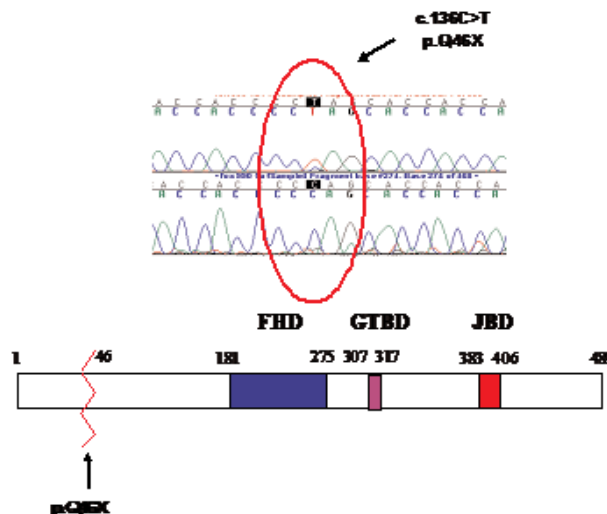




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Molecular analysis of FOXP1 in a series of 18 Italian patients

The FOXP1 gene has been recently implicated in the congenital form of Rett syndrome (RTT). It encodes the forkhead box protein G1, a brain-specific transcriptional repressor that is essential for early development of the telencephalon. Molecular analysis revealed that FOXP1 might also share common molecular mechanism with MeCP2 during neuronal development, exhibiting partially overlapping domain in postnatal cortex and neuronal subnuclear localization. I screened the entire coding sequence of FOXP1 for point mutations in a cohort of 18 Italian cases with a phenotype suggestive for congenital Rett. Using DHPLC followed by sequencing analysis we identified one de novo FOXP1 mutation: c.136C>T (p.Q46X). This mutation introduces a stop-codon (p.Q46X) that disrupts the protein at the N-terminal, causing the loss of the three main functional domains: the DNA binding fork-head domain (blue), the Groucho-binding domain (violet) and the JARID1B binding domain (red). Concerning the phenotype the patient shows atypical clinical features of the congenital variant. In fact, The girl presents milder neurological features respect to the previously reported patients, being able to walk independently at the age of 12 years. Actually stereotypical hand movements are not present, however they have been referred in the past. This new case underlines that there is genetic heterogeneity associated with FOXP1 mutations and it might contribute to better define genotype-phenotype correlation in Rett patients.



Part of this work is published in:
Mencarelli M, et al. Novel FOXP1 mutations associated with the congenital variant of Rett syndrome. J Med Genet. 2009 Jul 2.

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RB1 mutation analysis in 23 patients affected by retinoblastoma

Retinoblastoma (RB, OMIM#180200) is the most common intraocular malignancy of childhood, caused by a two-step inactivation of both alleles of RB1 gene. Patients with RB1 mutations in heterozygous state show variable phenotypic expression. Patients may develop tumors in both eyes or in one eye only (variable expressivity), while some individuals show no retinoblastoma at all (incomplete penetrance). RB1 mutations are spread throughout the gene including the promoter, most exons, and splicing regions of introns. Literature data about genotype- phenotype correlation, indicate that large deletions, nonsense or frameshift mutations causing premature stop codon, are associated with hereditary retinoblastoma. On the contrary missense or splice mutations are associated with unilateral retinoblastoma or incomplete penetrance. Using a combination of MLPA (multiple ligation probe-dependent amplification), DHPLC (denaturing high performance liquid chromatography) and direct sequencing analysis, we investigated a group of 23 patients: 10 bilateral cases (9 sporadic and 1 familial) and 13 unilateral cases (12 sporadic and 1 familial). Among 10 bilateral cases, we found 9 mutation with a detection rate of about 90%. Mutations consist in one frameshift, four nonsense and two splice mutations. Moreover two genomic deletion, one involving exon 24 and one involving exon 20, have been found. Among 13 unilateral cases, we identified four mutations (4/13; 30%): two nonsense mutation, one whole-gene deletion and one duplication involving exon 20. Despite studies on large cohorts suggest a genotype-phenotype correlation, our results indicate that the background for genotype-phenotype correlation remains an unsolved problem. Probably, second mutational events or participation of other modifier genes (acting positively or negatively) could contribute to the modulation of the RB phenotype.

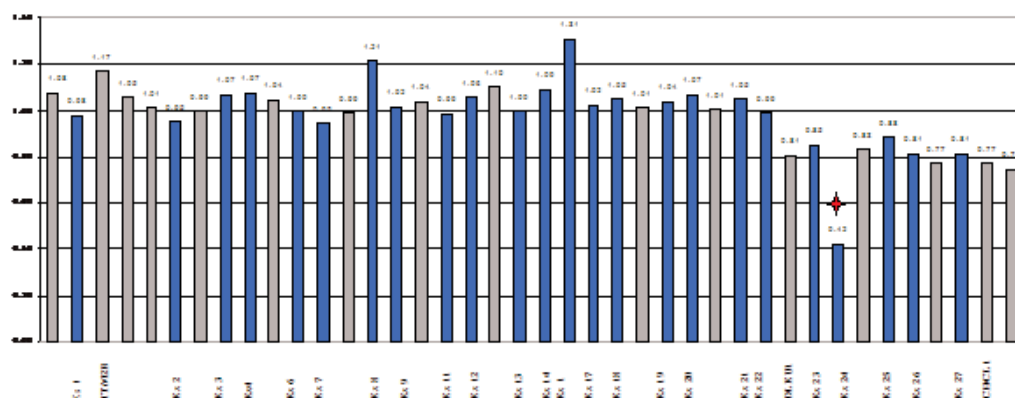


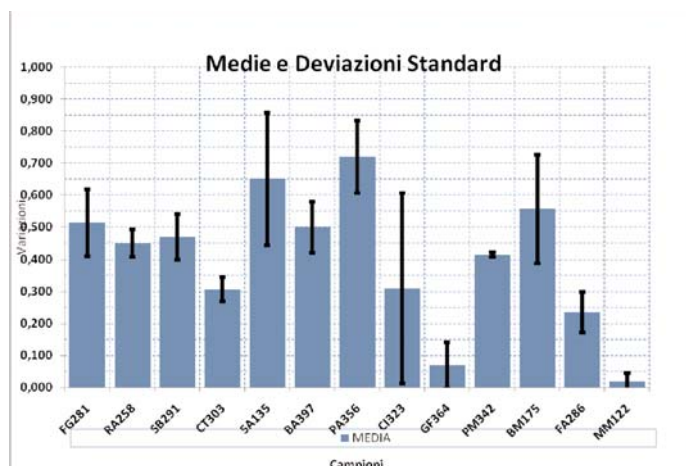
Fig. 1: MLPA analysis in one bilateral case shows deletion of exon 24.



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Gastric cancer and cell cycle regulation

Gastric cancer is still the second most common cause of cancer-related death worldwide. The highest incidence rates of such neoplasia are reported in Eastern Asia, while it seems to be decreasing in Western Europe. At present, the most reliable prognostic factors are grade and histological type. As far as concerns the histological characterization, the most important classification is the one proposed by Lauren ; according to him, there are two types of gastric cancer: intestinal- type and diffuse- type. The former is characterized by a series of well-defined steps beginning with atrophic gastritis and finally developing into carcinoma and diffuse metastasis. On the other hand, the latter has no well-defined characterization but a frequent initial chronic gastritis mainly due to Helicobacter Pylori infection; this type of carcinoma shows a worse prognosis. Since molecular bases of gastric cancer are so far not clear, the purpose of our work is focused on analyzing the role exerted by pRb family proteins, which showed a key role in the development of other kinds of tumors. The pRb family proteins is composed of three proteins: pRb/p105, pRb2 /p130 and p107. They regulate growth processes and apoptosis above all by interacting with E2F's transcription factors. Pocket proteins are phosphorylated by cyclin/cdk complexes, during cell cycle; this process allows the release of E2F factors and the transcription of gene involved in cell cycle progression. Our purpose is to characterize function, interaction and possible alteration of pocket proteins both in gastric cell lines and in tissues coming from patients with gastric cancer. So far we evaluated by real time PCR, Western blotting and Immunohistochemistry pRb2/p130 expression (gene and protein). At the same time we are analyzing pRb/p105 and p107 by Western blotting in cell lines. We are trying to define how the concerted activity of the three pRb family proteins maybe involved in gastric cancer development. Until now we found that pRb2/p130 shows gene down-regulation often associated with protein normal expression. Since both in tissues and in cell lines pRb2/p130 shows a nuclear localization. Our future purpose are verifying its function may be compensated or not by pRb/p105 and p107.



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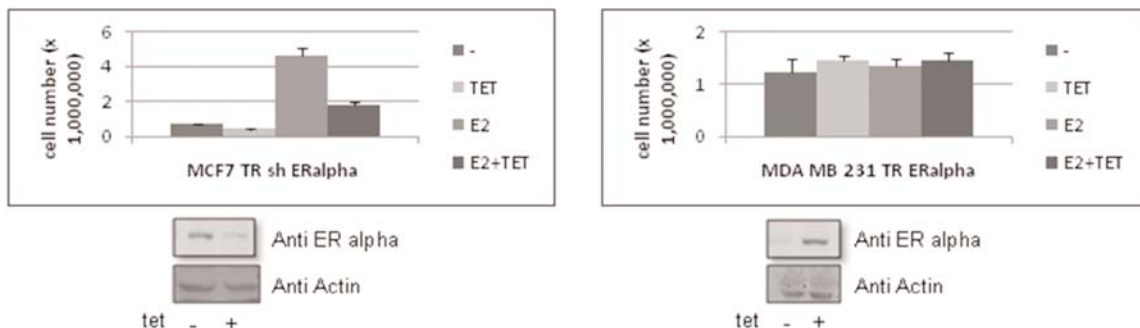


Characterization of the retinoid signaling pathway in breast cancer

Vitamin A regulates diverse cellular processes, including growth, differentiation and apoptosis. Natural and synthetic retinoids are promising compounds in the treatment and prevention of cancer.

Substantial pre-clinical evidence indicates that retinoids are useful in the treatment and chemoprevention of breast carcinoma. One of the main prognostic and progression determinants of breast cancer is the estrogen receptor (ER). ER influences the sensitivity of malignant cells to the anti-neoplastic activity of retinoids. ER-positive breast cancer cells are generally sensitive, while the ER-negative counterparts are refractory to the anti-tumor activity of the prototypic retinoid, all-trans retinoic acid (ATRA). It has been proposed that there is substantial cross-talk between the ER- and the retinoid-dependent intracellular pathways, however, the details of the interaction are not yet known.

The major aim of the research program of my PhD is to define the molecular mechanisms and determinants responsible for the ER-dependent regulation of retinoid sensitivity/resistance in breast carcinoma. For this purpose, during this year, we have established two complementary models of conditional ER over-expression and silencing using paradigms of ER-positive (MCF7) and ER-negative (MDA-MB231) breast cancer cells. MCF7 respond to ATRA with growth inhibition, differentiation and apoptosis, in contrast, MDA-MB231 are completely refractory to retinoid challenge. MCF7 have been engineered for the conditional expression of a short-hairpin RNA silencing ER after tetracycline treatment. By converse, we have introduced a tetracycline-dependent ER cDNA construct in MDA-MB231. To assess the role of ER signalling in the growth of each cell line, experiments have been conducted in the presence or absence of 17 β -estradiol (E2) (showed in the picture below). We have also take advantage of SKBR3 breast cancer cell line, a well known exception to the paradigm described above, since it is ER-negative but ATRA sensitive. The models described has been characterized for ATRA sensitivity allowing us to perform gene expression- and miR- microarray analysis for the dissection of the molecular mechanisms underlying the cross-talk between ER and the retinoid signaling pathways.





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Clinical Impact of Contemporary molecular cytogenetics

We investigated 332 MCA/MR patients. Forty six cases (14%) were considered positive using the following criteria: i) de novo non polymorphic rearrangements (5%); ii) inherited (15q11.2q13.2) or de novo rearrangements of known syndromes (6.6%); iii) inherited or de novo rearrangements in susceptibility regions (15q13.3/16p11.2) (1.8%), iv) inherited or de novo rearrangements with possible effects in surrounding genes (0.90%). The de novo non polymorphic rearrangements include: del2q24.3q31.1, del2q31.2, del3q27.3, del6p25.3, del6q24.3, del6q27, dup7q11.23, del7q22.1, del7q36.1, dup8q22.3, del9q31.1, del14q12, del14q32.31, del21q22.11 and a complex rearrangement of a del9p24.1 and a dup17p13.3. The group of known syndromes includes 1 case of 1p-, the shortest 4p- and 6q- known, 2 cases of Williams-Beuren syndrome, 1 case of a AS/PWS and 2 cases of AS/PWS reciprocal duplication, 3 cases of Potocki-Lupski, 2 cases of Smith-Magenis, 4 cases of del22q11 and 2 cases of dup22q11, 1 case of del22q13, 2 cases of dupXq28. Susceptibility regions include 3 dup15q13.3, 1 dup16p11.2 and 2 del16p11.2. We re-analyzed our cohort paying attention to genes lying up to 10Mb from the breakpoints. We describe two cases with a possible autosomal recessive syndrome due to a gene outside the rearranged region. In a sex reversal 46XX male a 1.8Mb inherited dup17q12 lays 4Mb upstream of the HSD17B17 gene that encodes a protein that catalyzes the final step of testosterone biosynthesis. In a sex reversal 46XY female a 0.2Mb inherited del17q12 lays 7Mb apart upstream of the above mentioned gene. This study allowed the characterization of several chromosomal imbalances in patients with complex phenotype, confirming the power of the array-CGH method.

Part of this work is published in:

- Pollazzon M, Grosso S, Papa FT, Katzaki E, Marozza A, Mencarelli MA, Uliana V, Balestri P, Mari F, Renieri A. A 9.3 Mb microdeletion of 3q27.3q29 associated with psychomotor and growth delay, tricuspid valve dysplasia and bifid thumb. *Eur J Med Genet.* 2009 Mar-Jun;52(2-3):131-3.
- Mencarelli MA, Kleefstra T, Katzaki E, Papa FT, Cohen M, Pfundt R, Ariani F, Meloni I, Mari F, Renieri A. 14q12 Microdeletion syndrome and congenital variant of Rett syndrome. *Eur J Med Genet.* 2009 Mar-Jun;52(2-3):148-52.
- Katzaki E, Morin G, Pollazzon M, Papa FT, Buoni S, Hayek G, Andrieux J, Lecerf L, Popovici C, Receveur A, Mathieu-Dramard M, Renieri A, Mari F, Philip N. Further delineation of the phenotype of the 21q22.11q22.12 deletion encompassing the RUNX1 gene. Submitted to the *Eur J Hum Genet*

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miRNA Expression Profiling in Malignant Mesothelioma

Malignant mesothelioma (MM) is a highly aggressive tumour that arises from the serous membranes of pleura, peritoneum, tunica vaginalis of testis and pericardium. The median survival from presentation of the disease is 9–12 months and long-term survival is rare even with aggressive multimodal therapy. At present there is no known curative modality for MM.

miRNAs are small noncoding RNAs that can effectively reduce the translation of target mRNAs and now are recognised as one of the major regulatory gene families .

In this research project we have analysed the miRNA expression profiling and the eventual abnormal expression of the miRNoma in patients with malignant mesotheliomas representative of the three different histotypes. We have selected 14 paraffin-embedded tumour tissue samples, and 4 samples of normal mediastinal pleura. Total RNA has been extracted from macrodissected tissue samples and has been analysed using miRNA microarray by Exiqon (miRCURY™ LNA Array Version 11.0). The miRNA profiling identified a subset (54), out of the total number of miRNAs analyzed by the miRCURY™ arrays, that are differentially expressed between normal and tumour samples. The results will be confirmed by real-time quantitative RT-PCR. For the miRNAs, whose expression will be found significantly up-regulated or down-regulated in MM, we will perform an 'in silico' analysis to identify the possible target genes corresponding to each miRNA.

At the same time, we started a project aimed at identifying potential therapeutic targets for malignant mesothelioma. One possible target could be Src family of non-receptor tyrosine kinases (SFK), since recent studies have indicated that deregulated activity of SFKs is an important factor in the growth, progression and metastasis in a number of primary tumours and tumour cell lines derived from patients with different types of cancers. Several Src inhibitor molecules have been synthesized and their growth inhibition capacity has been tested on different types of tumour derived cell lines. So we decided to analyze the effects of four new molecules, as c-Src inhibitors, on mesothelioma cell lines.

We have assessed the antiproliferative effect of these molecules on four neoplastic cell lines of malignant mesothelioma , in which the activated form of c-Src (p-Src y419) has been shown by western blot, and one non-neoplastic mesothelial cell line , used as a control. Cell proliferation has been monitored using MTS (tetrazolium salt/formazan method) assay. Our results demonstrate a marked decrease of the proliferation rate of neoplastic cell lines after the treatment with different concentrations of c-Src inhibitor molecules. Conversely, no cytotoxic effect was seen for the non-neoplastic cell line. The inactivation of c-Src has been proved by western blot on protein extracts from treated cell lines. In order to characterize the mechanisms of cell death induced by c-Src inhibitor molecules, cytofluorimetric analysis will be conducted.



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Pollution related inflammatory and malignant diseases in subjects living in Milan, in particular, concerning the possible role of infection and airway Ph

Environmental pollution is one of the great challenges of metropolitan areas. Particulate material (PM₁₀, PM_{2,5}) has been associated with adverse health effects both on the air tract and cardiovascular system. In fact, even if PM is likely to cause health damage through a wide range of pathophysiological mechanisms: 1. A common mechanism for all inhaled of xenobiotics is the generation of free radicals via oxidative stress; 2. Different PM components have variable potency and inflammatory efficacy, with organic nanoparticles originating from incomplete combustion or traffic related showing the highest potency; 3. The final clinical outcome is based on individual susceptibility, including both genetic predisposition and previous personal history.

In the City of Milan, from two consecutive years two groups of two sub-groups of patients (one group with children living in Milan City and the other one with older than 65 years, living in “hospices for detired peoples”), have been recruited and compared, with the aim of evaluating cardiovascular and respiratory side effects of environmental pollution, in particular of pollutants related to urban traffic. For the first time, last year in Milan City our team has performed visits and instrumental examinations on the young people living and working in the city (working in bancs, institutions, offices), within aims to have data information about normal population, living and working in Milan city. This supplementary adjustment was corrected and added in this big project the last year to gave more exactly information and to understand and simplefy the significance of two different situation, normal population in clean areas and at risk population (not only detired people in the hospices of Milan City) and those respectively in the principal polluted areas of the Lombardy. Clinical, laboratory and instrumental data were compared with particle number concentration and qualitative analysis of PM, measured “in situ”. i.e both outdoor (residence garden) and indoor (common places inside the building), in order to have direct measure of PM concentration during the same period as clinical evaluation. In addition to questionnaires concerning previous personal history and clinical examination, instrumental evaluation of the respiratory function by spirometry, NO measurement, BMI, EBC and laboratory examination (biochemical and genetic analyses) were performed, including blood samples and EBC analysis. From a preliminary study of biochemical data (presented by our team-CNR Niguarda-Policlinico di Milano in Bari (October 2008) and Genoa last September 2008 in the conference about PM pollution), we had a big point to clarify and understand significance between groups, for this reason we have sub-divided more and more each of the groups to gave homogeneous sub-groups and to profit a clear comparison and data with statistically significances in one year of the study. Oxidative stress determined by lung –pollutant reaction, could play a major role in the occurrence of adverse health effects in humans. Therefore, evaluation of reduced and total thiols, both in plasma and in red cells and, more in general, in biological fluids, could be of value for both detection of oxidative stress and early recognition of at risk subjects.

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Molecular dissection of BRD4 interaction with P-TEFb complex

The bromodomain-containing protein 4 (BRD4) is a member of the BET (bromodomains and extraterminal) protein family, a class of transcriptional regulators whose members are evolutionally conserved among animals, plants, and fungi. The BET proteins typically have two tandem N-terminal bromodomains followed by an ET domain. Brd4 is used as a cellular adaptor by some animal and human papillomaviruses (HPV) for anchoring viral genomes to mitotic chromosomes. In addition, Brd4 has been found in several transcription complexes, including the P-TEFb elongation factor. Proteomic analysis revealed that Brd4 interacts with cyclinT1 and Cdk9 that constitutes core positive transcription elongation factor (P-TEFb). Brd4 is a positive regulator of P-TEFb, a Cdk9-cyclin T1 heterodimer that stimulates transcriptional elongation by phosphorylating RNA polymerase II. Brd4 recruits P-TEFb to chromosomes at late mitosis to promote G1 gene expression and cell cycle progression. Starting from this important information, we are characterizing the interaction between BRD4, CDK9 and cyclin T1. Our goal is to understand the new function related to BRD4 and P-TEFb in the cell cycle control and to design a new pharmacological compounds able to modulate or inhibit Cdk9 activity.

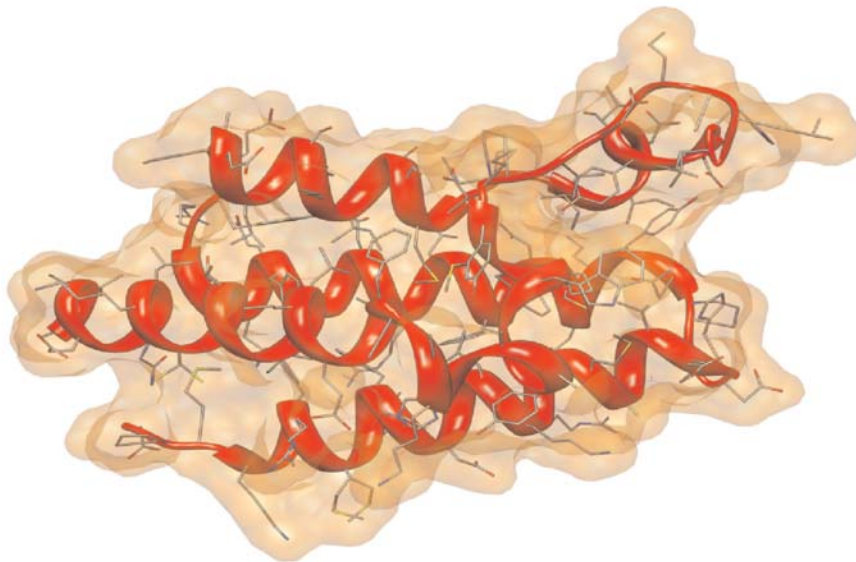


Fig. 1: Crystal Structure of the Bromo domain 2 in human Bromodomain Containing Protein 4 (BRD4).



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Air pollution from Milan affects “in vitro” sperm quality more in individuals with previous varicocele than in normal subjects or in rabbit sperm cells

The aim of the study has been to investigate “in vitro” if environmental pollution from Milan, in particular samples of PM 10 and PM 2,5 (Particulate Matter: a mixture of dust, dirt, soot or smoke and liquid droplets), may affects human and/or rabbit sperm quality.

Every day were measured levels of PM 10 and PM 2,5 by an O.P.C. detection unit, both outside primary school of children and two hospices for retired people in Milan.

In particular, an OPC 1.108 Dustcheck detector (Grimm) was used for indoor measurements, whereas as OPC 1.107 “Environcheck” model (Grimm) was used for outdoor analysis.

Aliquots of these samples at concentration of 10, 50, 75 µg/ml were incubated for 4 and 6 hours with rabbits spermatozoa and sperm cells from humans with normal quality and men with varicocele.

Were considered several parameters, including motility traits, to evaluate sperm characteristics before and after sperm exposure to PM.

Treatment with PM 2,5 at concentration of 10, 50, 75 µg/ml didn't show evident damage in rabbit spermatozoa when the samples were incubated for 4 and 6 hours.

On the contrary 50 µg/ml caused in human samples decrease in progressive motility and increase in the percentage of necrosis and apoptosis.

Samples from patients with abnormal semen parameters (varicocele) showed even worse semen quality.

In fact, they showed reduced semen quality already after 4 hours of incubation at a concentration of 10 µg/ml.

The mean + standard deviation of sperm motility was 11+ 4,08. Mean values of Fertility Index (FI) also were strongly reduced.

Moreover, Annexin V / Propidium iodide assay was carried out to quantify the presence of necrosis and apoptosis in all sperm samples from rabbits and men and it showed an increase in these percentages.

A further decrease of sperm quality (progressive motility decreasing from 20% to 4%, necrosis from 33% to 53% and FI from 726004,5 to 162500,25) was observed after 4-hour incubation at a concentration of 50µg/ml.

In conclusion present data “in vitro” showed a progressively greater damage to the reproductive function passing from rabbit sperm to normal human sperm and to sperm from men with altered semen.

Sperm alteration and PM related damage to the reproductive function could be suitable model to make comparison of health damage among various types and/or concentration of pollutants and/or various types of hosts.

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Short-term effects of chemoradiation therapy on internal anal sphincter: a human in vitro study

Neoadjuvant chemoradiotherapy (CRT) has been shown to reduce local recurrence and improve overall survival, and is recommended in the treatment of patients with locally advanced rectal cancer. Surgery alone adversely affects the quality of life of these patients, resulting in increased evacuation disorders and symptoms of faecal incontinence. However, there is accumulating evidence that the addition of CRT further worsens anorectal function. We investigated the functional changes of the internal anal sphincter (IAS) following CRT.

IAS strips collected from patients undergoing abdominoperineal resection or proctectomy were mounted in an organ bath and responses to electrical field stimulation (EFS) and drugs were monitored. Five patients were treated by surgery alone, and six received pre-operative CRT.

After equilibration, all strips developed basal tone, but CRT strips were less reactive. EFS caused initial relaxation, followed by a small contraction. At 50 Hz, EFS produced $47.0 \pm 6.2\%$ (mean \pm standard error) of maximum relaxation in control strips but only $25.6 \pm 4.9\%$ in CRT strips, followed by a contraction of $17.7 \pm 4.0\%$ vs $5.5 \pm 0.9\%$ of the basal tone in control and CRT strips respectively. Relaxation was significantly attenuated by N ω -nitro-L-arginine. Significantly smaller relaxations to carbachol and contractions to phenylephrine were found in CRT strips. Sodium nitroprusside caused similar relaxation in both groups.

CRT significantly impairs IAS function and intrinsic nerves seem more susceptible than smooth muscle. The exclusion of the anal canal from the radiation field is recommended, when oncologically safe.



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Intrinsic toxicity, inflammatory potency and individual susceptibility in the occurrence of health damage from Particulate Material (PM)

Once Particulate Material (PM) introduced by inhalation has reached pulmonary interstitial sites, uptake into the blood circulation, in addition to lymphatic pathways, can occur, depending on particle size, favouring nanoparticles. In particular, particle size, surface chemistry and possibly charge, govern translocation across epithelial and endothelial cell layers. Depending on particle surface chemistry, nanoparticles have been shown to transcytose across alveolar type I epithelial cells and capillary endothelial cells, but not via cellular tight junctions in the healthy state. However, in a compromised or disease state, translocation across wide and tight junctions occurs as well, because of functional cell membrane damage due to sepsis.

In particular, cross sectional and panel longitudinal studies have been performed, both in children and in old patients. Clinical, laboratory (respiratory exhalate) and instrumental data (spirometry), BAL (bronchioloalveolar lavage) and in some cases tissue biopsy, BAL culture and pH analysis, were compared with seasonal PM concentration and speciation and in vitro studies on cell lines, incubated with the same PM (PM 10, PM2,5, PM1), that was collected in the same site and period as data collection for clinical study.

Preliminary data showed that the most severe features and complications during PM peaks occurred in patients with previous long-lasting infections asthma, or COPD pulmonary.

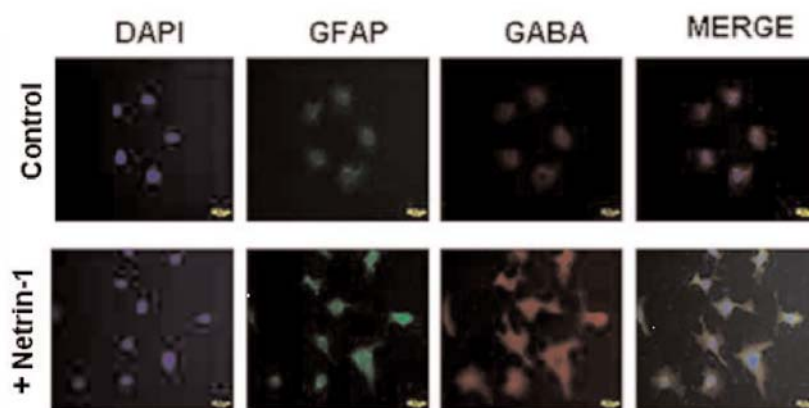
Overall data suggest that, in addition to individual susceptibility, due to genetic variability, which greatly affects health effects of host particle interactions, in the presence of the same PM concentration and exposure, the occurrence and severity of symptoms greatly varies among subjects with physiologic or pathologic airway conditions.

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Neuronal guidance protein Netrin-1 induces differentiation in human embryonal carcinoma cells

Pluripotent cells within embryonal carcinomas are known to differentiate in vivo or in vitro upon treatment with specific agents. Differentiated embryonal carcinoma (EC) cells express lower levels of pluripotential related genes like Cripto-1. Cripto-1 is a member of the epidermal growth factor(EGF)-CFC family and it has been implicated in embryogenesis and carcinogenesis. Cripto-1 represents one of several target genes of the stem cell-related transcription factors, Nanog and Oct4, which have been shown to be involved in the maintenance of self-renewal and pluripotency. Here, we show that migration of human EC cells (NTERA/2 and NCCIT) can be reduced following treatment with the guidance molecule Netrin-1. Moreover, Netrin-1 induced increased levels of beta-III tubulin, glial filament acidic protein (GFAP), Nestin and gamma aminobutyric acid (GABA) and reduced the levels of Cripto-1, Nanog and Oct4 in the treated EC cells. We found that the Netrin-1-induced effects in the EC cells was mediated via interaction with the Neogenin receptor and activation of the tyrosine phosphatase, SHP-2, resulting in increased levels of the inactive form of c-src, P-c-src(Y527). These results suggest that Netrin-1 can induce neuroectodermal-like differentiation of human EC cells by affecting c-src signaling pathway via SHP-2 activation resulting in the negative regulation of the expression of Nanog, Oct4 and Cripto-1.



Part of this work is published in:

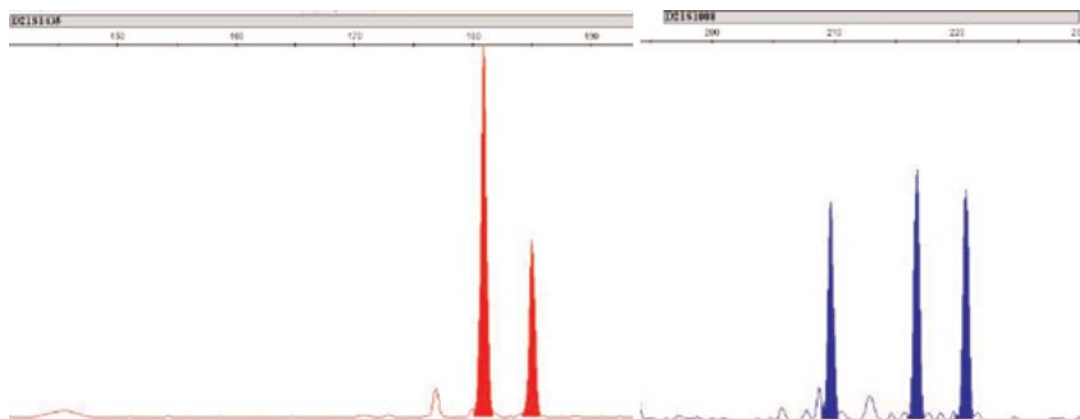
Mancino M et al. Neuronal guidance protein Netrin-1 induces differentiation in human embryonal carcinoma cells. *Cancer Res.* 2009 Mar 1;69(5):1717-21. Epub 2009 Feb 17



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Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR, one year of experience

Rapid prenatal diagnosis of major chromosome abnormalities can be performed on a large scale using Short Tandem Repeats (STRs) amplified by the Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR). Our objective was to investigate QF-PCR as a means for prenatal aneuploidy screening. The combination of markers included in the Aneufast QF-PCR Kit allows the detection of aneuploidies involving chromosomes 13, 18, 21, X and Y with 100% sensitivity and specificity, reporting results in 48h-72h. The most common indications for prenatal diagnosis in our cohort were advanced maternal age (91,8%), ecographic anomalies (4,5%) and biochemical risk (3,7%). In one year, we have analyzed 243 prenatal samples: 106 amniotic fluids and 137 chorionic villus. QF-PCR assays detected three cases of trisomy 21, one case of trisomy 13 and one case of triploidy, which were confirmed by traditional karyotyping. Our results are in accordance with recent studies, that following the analysis of several thousand samples, have shown that this rapid approach has a very high rate of success and it could reduce the need for cytogenetic investigations. The main advantages of the QF-PCR are its accuracy, speed, automation, and low cost that allows very large number of samples to be analyzed by few operators. For these reasons, in a near future, this kind of assay could replace cytogenetic analyses.



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BKV infection, inflammatory response and cancer stem cells: new actors in PC scenario

A great number of studies try to identify markers associated with the initiation and progression of prostate cancer (PC). Inflammatory cytokines could promote transformation during prostate inflammation and may act as a growth factor in response to androgen deprivation contributing to hormone refractory PC. The initial trigger for prostatic inflammation is unclear. Candidate sources include infection, cell injury and hormonal imbalance. In 2008, our group demonstrated that Human Polyomavirus BK (BKV) could act as a cofactor in the pathogenesis of PC evidencing that large T antigen (TAg) represents the major viral oncogenic protein. We found that TAg was localized along with the oncosuppressor p53 in the glandular epithelial cells' cytoplasm of PC sections, whereas, in the absence of TAg, p53 was nuclear. Therefore we have speculated that TAg, sequestering p53 into the cytoplasm, blocks its interaction with cellular proteins involved in the cell cycle arrest or apoptosis. Moreover, like many cancer viruses, BKV can shift host immune response from limiting viral infection and controlling the virus carrier state, to amplification of inflammation. It could promote the progression, cytokine-dependent, towards more advanced cancer stages. In the future this issue will be investigated. Finally, within prostate epithelium, stem cells may be a target for mutations since their longevity assures a continuous exposure to carcinogenic agents (carcinogenic viruses) that favour cancer development. Therefore the identification of cancer stem cells markers and the study of their inflammatory functions provide an useful novel tool for understanding PC molecular pathways and have important implications for pharmacological therapy.

Part of this work is published in:

Russo G, Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Giordano A, Autran-Gomez A, Di Monaco F, Di Silverio F, Sale P, Di Prospero L, Pietropaolo V. p53 gene mutational rate, Gleason score, and BK virus infection in prostate adenocarcinoma: Is there a correlation? *J Med Virol.* 2008 12:2100-7.

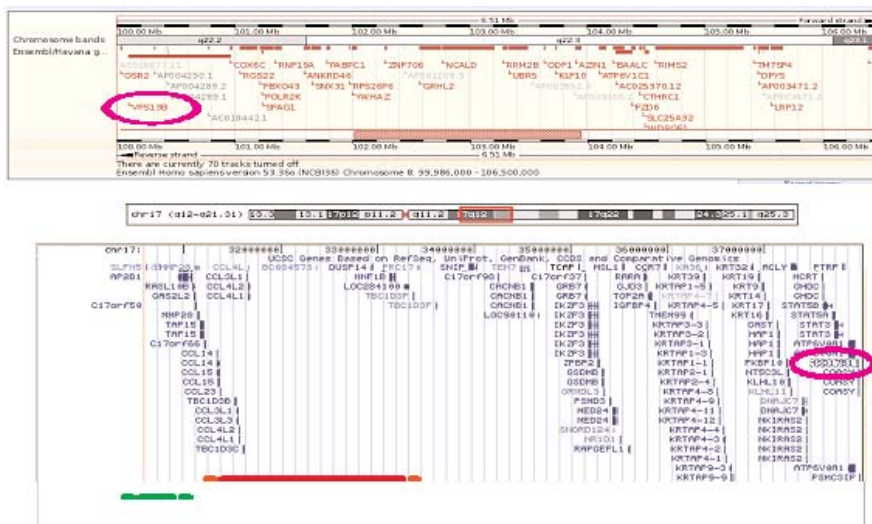
Fioriti D, Mischitelli M, Di Monaco F, Di Silverio F, Petrangeli E, Russo G, Giordano A, Pietropaolo V. Cancer stem cells in prostate adenocarcinoma: a target for new anticancer strategies. *J Cell Physiol.* 2008 Sep;216:571-5.



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Side effects of de novo or inherited microdeletion/microduplications

A cohort of 332 mentally retarded patients has been analyzed by array-CGH with a resolution of about 100Kb (Agilent 44K). Since it has been proved that rearrangements may alter expression of genes lying up to 10Mb from the breakpoints, we re-analyzed our cohort of patients paying attention to the surrounding regions. Here we describe three cases (1.4%) with a possible autosomal recessive syndrome due to genes outside the rearranged region. In a 15 years old male patient with 46 XX karyotype, array_CGH analysis identified a 1.8Mb duplication in 17q12. Analysis of the SRY gene resulted normal and deletions of WT1 and PAX6 genes were excluded. This rearrangement was present in the healthy father and in the sister with isolated behavioural problems. The duplication in 17q12 lays 4Mb upstream of the 17beta-hydroxysteroid dehydrogenase 1 gene. It encodes for 17HSD1, which catalyzes the final step of testosterone biosynthesis. Lately Saloniemi et al. have demonstrated that over-expression of human HSD17B1 results in female transgenic (TG) mice in masculinization of the external and internal genitalia. In a sex reversal 46XY female a 0.2Mb inherited deletion in 17q12 lays 7Mb apart upstream of the above mentioned gene. We propose that the 17q12 rearrangement includes regulatory elements that modify the expression levels of 17HSD1 and thus could be responsible for patients sex reversal phenotype. In a Cohen-like patient (microcephaly, truncal obesity tapered fingers with brachidactily, short stature, evocative facial gestalt) a 2.1Mb de novo deletion in 8q22 lays 1Mb downstream to the COH gene. In order to prove the hypothesis of recessive syndromes due to genes outside the rearranged region, we started to perform mRNA and mutation analysis on the two candidate genes.



Part of this work is published in:
 Katzaki E, Papa FT, Mucciolo M, Uliana V, Renieri A. Is HSD17B1 a new sex reversal gene in human? Mol Cell Endocrinol. 2009 Jul 27.

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Function of mir-128a in normal and in pathological conditions

MicroRNA (miRNA) are a growing class of short non-coding RNAs that control post-transcriptional gene expression. Mature microRNA derive from longer transcripts (pri-miR) which are processed to shorter hairpin precursor (pre-miRs) by the action of Drosha enzyme. The 70-nucleotides precursors are exported to the cytoplasm where they are cleaved by Dicer to produce mature 19-22 nucleotides microRNA, which enter RNA-induced silencing complex (RISC).

Translational silencing by the RISC complex appears to regulate a wide variety of cellular and developmental processing. An increasing number of studies have shown the presence of microRNAs in the central nervous system (CNS) and their importance for neuronal development. The enrichment of microRNAs in the neuronal processes suggests a possible action of dendritic microRNAs in regulating synaptic function.

Glioblastoma is the most frequent and one of the most aggressive brain tumors, which is characterized by rapid and infiltrative growth. Multiple genetic alterations are also typical hallmarks of glioblastomas, contributing to their heterogeneity and therapeutic resistance. Epidermal growth factor receptor (EGFR) amplification and PTEN mutations are typical for primary glioblastomas developing rapidly de novo. Recently, expression profiling of microRNAs has been utilized as a novel tool for molecular characterization of tumors, including brain tumors. Mir-128 belongs to brain specific microRNAs and it is downregulated in glioblastomas, in which is suspected to negatively regulate cell proliferation. We utilized LN229 glioma cell line (which have functional PTEN) and found that expression of mir-128a in these cells results in increased phosphorylation of the pro-survival factor Akt in the absence of serum. Further investigation will elucidate the role of PTEN and its mutations in the mir-128a-mediated activation of Akt.



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Array-CGH should replace traditional cytogenetic analysis for the identification of the genetic cause in miscarriages

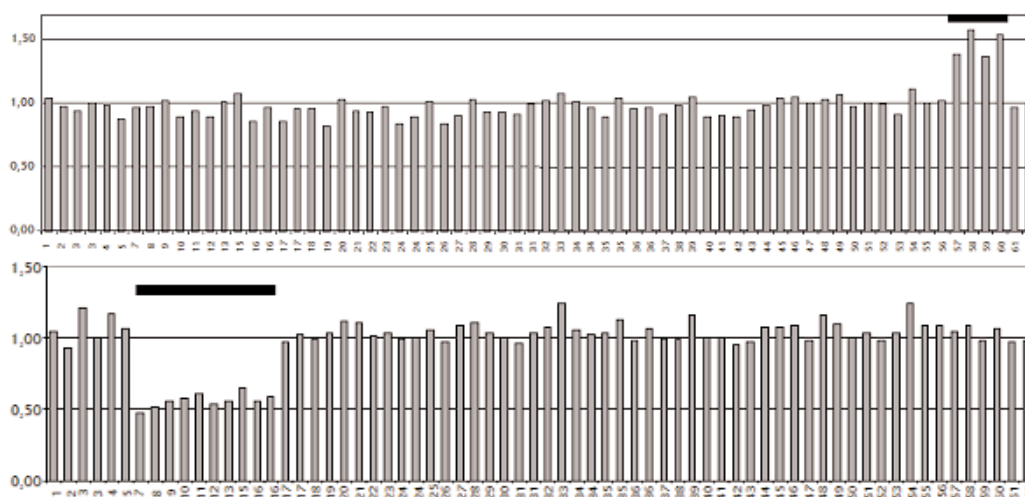
Recurrent miscarriages, defined as three or more clinically recognised spontaneous pregnancy losses occurring before 24 weeks of gestation, represent an important clinical problem with profound emotional impact, affecting up to 3% of women. Approximately 15% of clinically recognizable pregnancies result in spontaneous abortions. Around half of the first-trimester losses are caused by chromosomal abnormalities, the great majority of which are numerical aberrations. Presently in developed countries, the offered protocol is aimed to identify the causes of miscarriage, differentiating genetic from non genetic factors. This technique requires the culture of the abortive material but presents several drawbacks such as tissue culture failures, overgrowth of maternal cells over fetal cells, suboptimal quality of chromosome preparation, and the limit of resolution in conventional chromosomal banding. From 2005 onwards most karyotype analysis in postnatal diagnosis has been replaced by a more sensitive technique, array-CGH. This technique does not require cell culturing, can be applied even in cases of poor material if pre-amplified and has a higher resolution than karyotype. Here we report a pilot study on 21 miscarriages. We identified 5 complete trisomies: one on chr 8, 14, 21 and two on chr 22. Karyotype failed to detect trisomy on chr 14. Two mosaic monosomies on chr 19 and three complex rearrangements not seen by karyotype were also detected. We demonstrated that array-CGH is able to provide the result in 100% of cases. We proved that array-CGH is more sensitive than karyotype because it can detect the genetic defect also in cases where conventional cytogenetic has failed and highlight additional genetic aberrations.

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High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome

Cohen syndrome is a rare autosomal recessive disorder with variability in the clinical manifestations. It is characterized by mental retardation, postnatal microcephaly, facial dysmorphisms, ocular abnormalities and non-cyclic neutropenia. Mutations in the COH1 gene have been found in patients with Cohen syndrome from different ethnic origins but there is still a high percentage of patients where only one mutated allele or no mutated allele is detected. To verify if large rearrangements could account for missed COH1 mutations in a group of 14 patients with a clinical diagnosis of Cohen syndrome, we employed multiplex ligation-dependent probe amplification (MLPA). This analysis allowed us to identify 11 multi-exonic deletions and 4 duplications. To our knowledge COH1 intragenic duplications have never been reported in Cohen syndrome. Among the 11 deletions, 5 shared the same extension with one already reported in a large Greek consanguineous family, spanning from exons 6 to 16. The 3 duplications showed different locations spanning from exons 4 to 13, from exons 20 to 30, and from exons 57 to 60, respectively. The use of MLPA was therefore crucial in identifying mutated alleles undetected by traditional screening and in defining the extension of the rearrangements. In conclusion, MLPA demonstrated to be an essential tool for the detection of copy number variations within the COH1 gene at relatively low cost and thus we consider that it could be used as initial screening method for the molecular diagnosis of Cohen syndrome.



Part of this work is reported in:

Parri V. et al, High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome (submitted to the Eur J Hum Genet).



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Pin1 controls cell cycle progression through interaction with pRB

Normal cells became tumor cells through deregulation of multiple pathways. Much evidence suggests that each type of tumors involves different proteins so that each type of cancer cells is different from the others. Nevertheless, there are some pathways that are altered in many tumors and RB pathway is one of the most important. pRB controls the cell cycle through the interaction with E2F transcription factors. These interactions are regulated during cell cycle by a phosphorylation mechanism. Ser or Thr followed by Pro are major phosphorylation motifs in the cells but their significance was obscure until the discovery of the PIN1 protein (protein interacting with NIMA (never in mitosis A)-1).

Pin1 is an isomerase specific of pSer/Thr-Pro motifs that catalyzed the conformational switch from cis to trans, which is especially important because Pro-directed kinases and phosphatases are conformation-specific and act only on the trans conformation (. In vivo and in vitro data have demonstrated that Pin1 is involved in many aspects of cell cycle control. PIN1 was originally identified and defined as a protein that functions in mitosis. Since then, a plethora of protein targets have now been discovered many of which are involved in the G0, G1/S control. Here we show that Pin1 controls tumor cell proliferation through direct interaction with the pRB protein. The interaction is phosphorylation-dependent and it is also necessary for pRB phosphorylation. Finally, pRB is the major Pin1 target to control transition from the G1 to S phase of cell cycle.

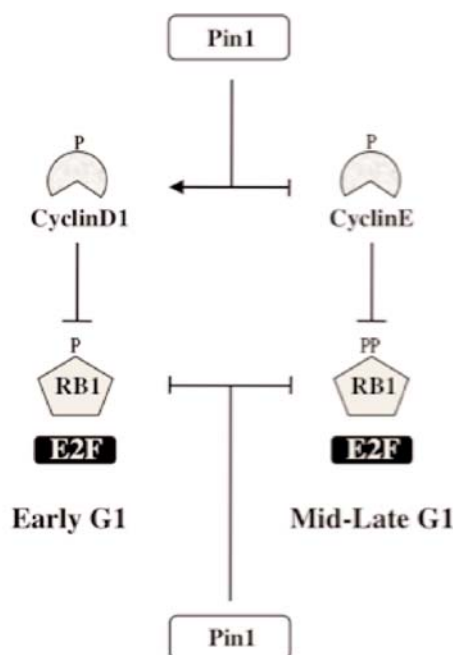


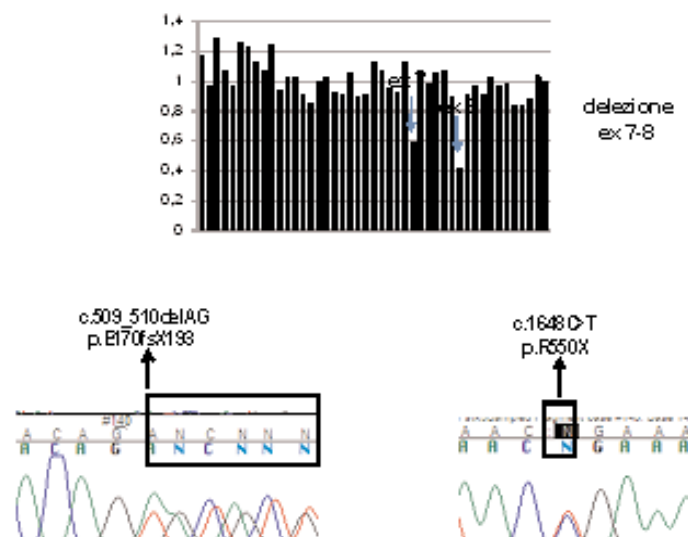
Fig. 1: A model of Pin1 and pRB interaction

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Identification of CDKL5 mutations in patients with the early seizure variant of Rett syndrome

Rett syndrome (RTT, OMIM#312750) is a severe neurodevelopmental disorder characterized by a wide spectrum of clinical manifestations. Among RTT clinical variants, the early onset seizure variant is caused by mutations in the CDKL5 gene (Xp22). In order to perform CDKL5 mutation screening in a group of 14 patients, we employed both DHPLC and MLPA. This diagnostic strategy led us to identify three de novo CDKL5 mutations. Mutations include two truncating point mutations (p.E170fsX193, p.R550X) and a large deletion encompassing exons 7 and 8. The frameshift mutation p.E170fsX193 (c.509_510delAG) and the large rearrangement are located within the kinase domain, while the stop mutation p.R550X (c.1648C>T) affects the C-terminal regulatory region of the protein. Concerning the phenotype, the three girls present drug resistant epilepsy with early onset. Patients do not show a classic regression period due to precocious timing of seizure onset. Psychomotor development is severely impaired. Patients present typical persistent stereotypic hand movements and acquired microcephaly. Considering these results and the phenotype of the previously reported patients (Artuso et al. Brain Dev. 2009), we suggest clinical criteria that will be of practical value in favoring the diagnosis of early-onset seizure variant of RTT. Early epilepsy, with an onset between the first week and 5 months of age, is the key feature. Additional characteristics include normal prenatal history and a quite normal perinatal period, severely impaired psychomotor development and normal somatic growth, poor eye contact, absence of speech and presence of stereotypic hand movements.





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Rett Syndrome: mutation analysis of MECP2 in a series of 21 patients

Rett syndrome (RTT) is an X-linked progressive neurodevelopment disorder that affects almost exclusively girls with an estimated prevalence of 1 in 10.000 to 20.000 female births. It represents a leading cause of mental retardation and autistic behavior in girls. In addition to the classic form, five distinct RTT variants have been described. Among RTT variants the Zappella variant is the most common in our series, while others such as “forme fruste” or the late regression variants are less frequently observed. MECP2 mutations account for approximately 90-95% of classic RTT cases and for a lower percentage of variants (20-30%). We performed MECP2 mutation screening in 7 classic RTT patients and 14 RTT-Like patients (detailed clinical information is collected in the Italian RTT database: <http://www.biobank.unisi.it>) by DHPLC (Denaturing High Performance Liquid Chromatography) for the detection of point mutations and by MLPA (Multiplex Ligation-dependent Probe-Amplification) for the identification of large rearrangements. For this last application, we used Salsa MLPA P015-C2 kit (MRC-Holland), containing probes for all MECP2 exons (1-4) and flanking genes (IRAK1, L1CAM and SYBL1). By DHPLC followed by sequencing we characterized 9 MECP2 point mutations: 5 nonsense, 2 missense, 1 splice site mutation and 1 frameshift C-Terminal deletion. By MLPA, we detected one MECP2 large deletion including exons 3 and 4. An accurate clinical re-evaluation will address future molecular test in mutation-negative RTT patients.

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Project of the European Database on Rett Syndrome

The aim of this project is to connect the already existing databases concerning the Rett Syndrome and to create a unified European repository. The European Rett database will allow to collect standardized and easily comparable clinical and genetic data of a huge number of Rett patients. The data will be accessible to the scientific community according to rules that assure transparency and equity. This international effort will be of great value in order to find out genotype-phenotype correlations, to study modifier genes, and to select subgroups of patients for clinical trials.

After the preliminary requirements' analysis, the main goal of my research is to develop a data mining system, which can manipulate large scientific databases. The overall approach will be that of identifying some basic data mining operations, which cut across applications, in order to develop scalable retrieval algorithms. Such algorithms should be able to discover patterns in large databases, show high performance, and be particularly tailored to "mine" genetic data, so that to extract possible unknown genotype-phenotype correlations. With particular reference to the last point, machine learning techniques, both in the supervised and unsupervised framework, will be employed in order to extract significant relationships, eventually allowing auto-organization among data.

Moreover, although successful in many applications, data mining poses special concerns for private data. Therefore, an integrated architecture must be devised that takes a systemic view of the problem, implementing established protocols for data collection, inference control, and information sharing (based on the European Union Privacy Directive mandate on privacy protection for data management and analysis systems).



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Partial silencing of Methyl Cytosine Protein Binding 2 (MECP2) in mesenchymal stem cells induces senescence along with increase of damaged DNA

Mesenchymal stem cells (MSCs) are of interest because of their multiple roles in the physiology of organisms. In fact, besides the differentiation in mesenchymal tissues, such as bone, cartilage and fat, they are capable of differentiating also in non-mesenchymal lineages, such as neurons and glia.

Several evidences demonstrate that ageing is in part associated to stem cell senescence that occurs as results of extrinsic or intrinsic agents that cause DNA damages. Ageing of MSCs can have profound consequences on the body physiology. For these reasons, in depth studies are needed to know the molecular events leading to impairment in MSC function.

Among regulators of chromatin status the MECP2 protein plays a key role by binding methylated CpG dinucleotides. MECP2 protein mediates gene silencing by causing changes in chromatin structure. Although it was considered a global transcriptional repressor, recent studies have provided evidences that MeCP2 function extends beyond gene silencing. In fact, MECP2 may be considered a transcriptional modulator rather than a transcriptional repressor. Rett syndrome (RTT) is one of the most common genetic causes of mental retardation. Mutations in MECP2 gene are found in up to 90% of classic RTT patients. In RTT patients MECP2 inactivation can impair epigenetic mechanisms regulating stem cell biology this in turn could alter the physiological development of tissues and organs.

For these reasons, we decided to investigate the biology of bone marrow MSCs in RTT patients in order to verify if mutation in MECP2 gene can result in alteration of stem cell biology (Squillaro et al., 2008). Our studies evidenced that MSCs from RTT patient showed precocious signs of senescence compared with healthy controls.

To confirm and extend this research we took advantage of adeno-siRNA technique to silence MECP2 in MSCs from healthy donors. Downregulation of MECP2 induced a decrease of cell proliferation and apoptosis along with trigger of senescence in MSC cultures. Reactive oxygen species (ROS), which are normal byproducts of cell's metabolism, are a chronic persistent damaging agent that greatly contributes to aging. 8-oxo-2'-deoxyguanosine (oxo8dG) that increases during cellular senescence is the major product of ROS action. Partial silencing of MECP2 augmented significantly the percentage of oxo8dG positive MSCs.

Senescence induced by partial silencing of MECP2 appears to rely upon impairment of DNA damage repair mechanisms. In fact, following transduction of MSCs with Ad-siRNA-MECP2, we observed a downregulation in the expression of several genes belonging to base and nucleotide excision repair, mismatch repair, and double strand break repair. In agreement with this result, we observed a reduced ability of MSCs transduced with Ad-siRNA-MECP2 to repair double strand breaks.

Cell cycle arrest and senescence induced by MECP2 silencing appear to be governed by activation of RB pathway. In fact, following transduction of MSCs with Ad-siRNA-MECP2, we detected an upregulation of RB gene expression along with a decrease of hyperphosphorylated inactive form of RB2/p130. The activation of RB-pathways seems to be confirmed by the observation that the cyclin kinase inhibitor P16INK4A, which prevents RB phosphorylation and inactivation, showed an upregulation of mRNA and protein expression.

Part of this work is reported in:

Squillaro T et al. Partial silencing of Methyl Cytosine Protein Binding 2 (MECP2) in mesenchymal stem cells induces senescence along with increase of damaged DNA (in submission)

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 Tutor M. Genuardi



Influence of TYMS expression and genotype on the clinical outcome of colorectal cancer patients treated with 5-fluorouracil

Thymidylate synthase (TS) expression levels appear to be related to response to 5-fluorouracil-based chemotherapy in colorectal cancer patients. Three polymorphisms have been proposed as modulators of TS mRNA transcriptional and translational efficiency: a tandemly repeated sequence (2R/3R) in the 5' UTR, a SNP within the 3R allele and a 6 bp deletion in the 3' UTR.

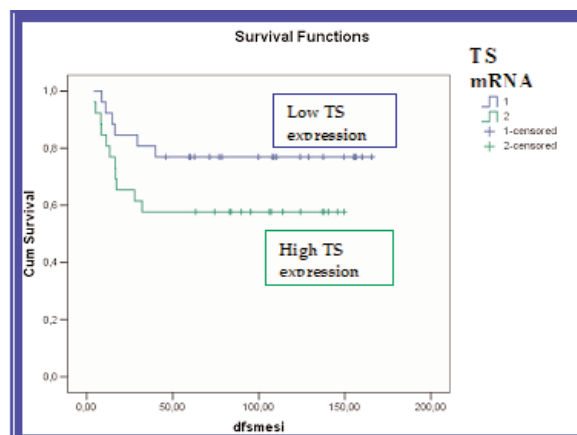
Our aim was to evaluate the influence of TYMS expression and genotype on the clinical outcome of patients treated with 5-FU-based chemotherapy.

TS expression levels were analyzed in healthy and tumor tissues, the coding sequence and 5' and 3' UTR genotypes were determined on genomic DNA from normal mucosa. In addition, LOH analysis was performed to determine the genotype of tumor tissues.

A difference in disease-free survival (DFS), although not statistically significant, was observed between high and low TS expression levels: patients with low mRNA levels showed longer DFS. This finding is in keeping with results obtained in most other studies.

No significant associations were observed between all polymorphisms analyzed and TS expression. The analysis of allelic combinations at the polymorphic sites showed the presence of linkage disequilibrium between 2R/3R and 6 bp deletion polymorphisms: the 3RG allele was associated with the 6 bp deletion, and, on the other hand, the 2R allele showed an association with absence of the 6bp deletion.

Since the alleles associated on these haplotypes exert opposite effects, this observation may explain the lack of association between TYMS genotype and mRNA levels. These results are in agreement with the growing evidence that the control of TS may require multiple mechanisms acting in close coordination with one another and suggest that TYMS genotyping alone is not sufficient to accurately predict response to 5-FU.





Oncology and Genetics Doctoral School
Hepatobiliopancreatic Disease and Multitumoral syndromes
XXIV cycle
Rosalia Zangari, BS
rosaliazangari@libero.it
Tutor F. Cetta

Detection of Cell Homeostasis Imbalance in two populations subjects at different exposure to Traffic Related Air Pollution

This study has been focused on the evaluation the role of thiol – cysteine (Cys), cysteinylglycine (CG), homocysteine (Hcy) and glutathione (GSH), with the aim of evaluating cardiovascular and respiratory adverse effects of environmental pollution.

Normal levels of thiols in physiologic fluids are considered as markers of good homeostatic equilibrium; their alteration has been associated with various diseases, usually related with increased production of reactive oxygen species (ROS). Air pollution promotes ROS generation and oxidative stress, causing imbalance of the antioxidant endogenous system. Therefore, levels of reduced and oxidized forms of thiols in plasma, in red cells and, more in general in all biological fluids - including exhaled breath condensate (EBC)- is considered a good marker of ROS activation and an early marker of related diseases.

In a polluted city, such as Milan, two groups of patients (n= 38, age $82 \pm 9y$), living in “hospices for retired peoples”, located within traffic crossroads and in a park, have been recruited and compared with 35 subjects (age $70 \pm 8y$) living in Aprica, a remote alpine site (1118 m. a.s.l.). We measured spirometry, fractional exhaled nitric oxide (FeNO) and we collected the information on previous clinical history.

Daily ambient concentrations levels of PM (10, 2,5 , $1 \mu m$) were measured both by OPC detectors and low volume gravimetric detectors.

HPLC analysis on blood (plasma total and reduced forms PT, PR respectively; erythrocytes total and reduced forms ET, ER respectively) and EBC samples were performed for the evaluation of thiols levels.

In addition, oxidative stress state was also evaluated by isoprostane (8-iso-PGF₂alfa), a lipid peroxidation index, by LC-MS/MS method.

There was no evident difference in the GSH levels between the two populations. On the contrary, there were same significant differences in the others thiols levels. These results support the hypothesis that, both in intracellular and extracellular fluid, dynamic equilibrium of thiols is able to preserve reduced GSH.

The present study of thiol redox equilibrium showed that Thiols can be monitored in all biological fluid, giving different values between more severely exposed and less exposed subjects, and could be used as markes of exposure and/or altered redox equilibrium, and /or oxidative stress and could be used for early detection of subjects who are greater risk for adverse health effect from air pollutants.

Thesis discussion Oncology and Genetics Doctoral School October 1, 2009 Centro Didattico S Maria alle Scotte, room 15

8.55 Entering of the PhD dissertation board composed by

- Prof. Alessandra Renieri (President),
Professor of Medical Genetics, University of Siena, Italy
- Prof. Enza Maria Valente (Member),
Professor of Medical Genetics, University of Messina, Italy
- Prof. Umberto Galderisi (Secretary)
Professor of Molecular Biology, Second University of Napoli, Italy

9.00 Thesis discussion in English language

- "Analysis of fetal nucleic acids (DNA and RNA) in maternal plasma for non-invasive prenatal diagnosis in genetic diseases and monitoring of pregnancy complications", Vincenza Causarano, XX cycle

9.30 Awarding of the PhD degree in Medical Genetics

11.00 Entering of the PhD dissertation board composed by

- Prof. Umberto Galderisi (President),
Professor of Molecular Biology, Second University of Napoli, Italy
- Prof. Enza Maria Valente (Member),
Professor of Medical Genetics, University of Messina, Italy
- Dr. Michele Zappella (Member),
Child Neuropsychiatrist, Siena
- Prof. Alessandra Renieri (Secretary)
Professor of Medical Genetics, University of Siena, Italy

11.05 Thesis discussion in English language

- "New perspectives on Rett Syndrome: The role of MECP2 gene in cellular senescence and neural differentiation", Tiziana Squillaro, XXI cycle

11.30 Awarding of the PhD degree in Medical Genetics

A copy of the thesis is available at http://www.unisi.it/ricerca/dottorationweb/genetica_medica/ accessing the "PhD student" link and then "PhD student in Medical Genetics".

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.

Doctor Europaeus Oncology and Genetics Doctoral School October 1, 2009 Centro Didattico S Maria alle Scotte, room 15

9.30 Entering of the PhD dissertation board composed by:

- Prof. Alessandra Renieri (President),
Professor of Medical Genetics, University of Siena, Italy
- Prof. Enza Maria Valente (Member),
Professor of Medical Genetics, University of Messina, Italy
- Prof. Hans Van Bokhoven (Member),
Professor of Human Genetics, Radboud University Nijmegen, The Netherlands
- Dr. Michele Zappella (Member),
Child Neuropsychiatrist, Siena
- Prof. Umberto Galderisi (Secretary)
Professor of Molecular Biology, Second University of Napoli, Italy

Thesis discussion in English language

- "Strategies for identification of new mental retardation genes", Rosangela Artuso, XXI cycle

9.50 Evaluation of candidate for qualification of Doctor Europaeus

The PhD dissertation board took into account the report of the following external PhD theses reviewers:

- Prof. Laurent Villard, Inserm Unit 910, Faculte de Medicine La Timone, Marseille, France
- Prof. Angus Clarke, Medical Genetics, University of Wales, UK
- Prof. Tayfun Ozcelik, Human Genetics, Bilkent University, Ankara, Turkey

10.00 Thesis discussion in English language

- "Clinical impact of contemporary molecular cytogenetics", Eleni Katzaki, XXI cycle

10.20 Evaluation of candidate for qualification of Doctor Europaeus

The PhD dissertation board took into account the report of the following external PhD theses reviewers:

- Prof. Guy Froyen, Dept. of Human Genetics, Catholic University of Leuven, Belgium
- Dr. Lina Florentin-Arar, Molecular Biology and Cytogenetics Center, Athens, Greece

10.30 Entering of the PhD dissertation board composed by:

- Prof. Antonio Giordano (President),
Professor of Human Pathology, University of Siena, Italy
- Prof. Enza Maria Valente (Member),
Professor of Medical Genetics, University of Messina, Italy
- Prof. Gerry Melino (Member),
Professor of Molecular Biology, Leicester University, UK
- Prof. Umberto Galderisi (Secretary)
Professor of Molecular Biology, Second University of Napoli, Italy

10.35 Thesis discussion in English language

- “Neuronal guidance protein Netrin-1 induces neuroectodermal-like differentiation by regulating the expression of stem cell markers Nanog, Oct4 and Cripto-1”, Mario Mancino, XXI cycle

10.55 Evaluation of candidate for qualification of Doctor Europaeus

The PhD dissertation board took into account the report of the following external PhD theses reviewers:

- Prof. Lionel Larue, Institut Curie, CNRS UMR 146, Centre Universitaire, ORSAY Cedex, France.
- Prof. Wolfgang Marek, Dept. of Physiology, Ruhr-University, Bochum, Germany.

11.00 Awarding of the PhD degree and qualification of Doctor Europaeus

A copy of the thesis is available at http://www.unisi.it/ricerca/dottorationweb/genetica_medica/ accessing the “PhD student” link and then “PhD student in Medical Genetics”.

PhD degree in Medical Genetics

October 1, 2009 Centro Didattico S Maria alle Scotte, room 15



From left to right:
Prof. Alessandra Renieri,
Prof. Enza Maria Valente,
Vincenza Causarano,
Prof. Umberto Galderisi.



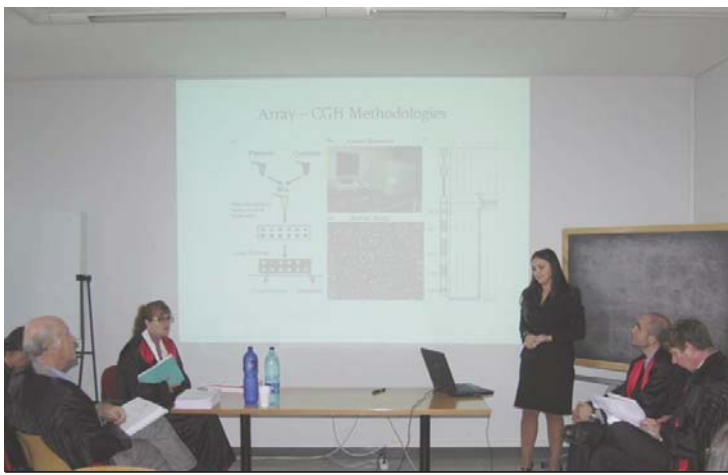
From left to right:
Dr. Michele Zappella,
Prof. Hans Van Bokhoven,
Prof. Alessandra Renieri,
Prof. Enza Maria Valente,
Tiziana Squillaro,
Prof. Umberto Galderisi.

Doctor Europaeus and PhD degree in Medical Genetics

October 1, 2009 Centro Didattico S Maria alle Scotte, room 15



From left to right:
Prof. Alessandra Renieri,
Rosangela Artuso,
Prof. Umberto Galderisi,
Prof. Hans Van Bokhoven.



From left to right:
Dr. Michele Zappella,
Prof. Enza Maria Valente,
Prof. Alessandra Renieri,
Eleni Katzaki,
Prof. Umberto Galderisi,
Prof. Hans Van Bokhoven.

Doctor Europaeus and PhD degree in Oncological Genetics

October 1, 2009 Centro Didattico S Maria alle Scotte, room 15



From left to right:
Dr. Michele Zappella,
Prof. Hans Van Bokhoven,
Prof. Alessandra Renieri,
Mario Mancino,
Prof. Umberto Galderisi,
Prof. Gerry Melino.

Phd students and examination board

October 1, 2009 Centro Didattico S Maria alle Scotte, room 15



From left to right:
Mario Mancino,
Dr. Michele Zappella,
Rosangela Artuso,
Prof. Hans Van Bokhoven,
Prof. Alessandra Renieri,
Prof. Enza Maria Valente,
Eleni Katzaki,
Prof. Umberto Galderisi,
Tiziana Squillaro,
Prof. Gerry Melino,
Vincenza Causarano.

