

PhD Students' works

Annual Progress Report 2006-2007

Oncology and Genetics
Doctoral School



University of Siena

Annual Progress Report 2006-2007

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

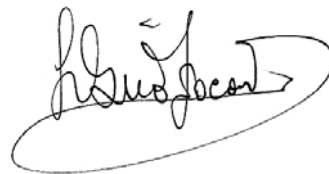
S.H.R.O. Sbarro Health Research Organization

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



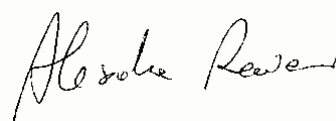
This pamphlet was created to regroup and present together the research activities of the students of the Doctoral School in Oncology and Genetics in order to exploit the work of the students and to promote the collaboration on research projects.

The first pages illustrate the activity of the “annual progress report day”. This day takes place at the end of each academic year and it 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 32 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 with their continuous daily work, their perseverance and motivation represent the “mainstay” of the Institution that we call University.

The director of the School
Prof. Alessandra Renieri



The Doctoral School in Oncology and Genetics belongs to the Scuola Superiore S. Chiara (<http://www.unisi.it/santachiara/>) and it is constituted of 4 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.

In addition to the four above mentioned coordinators, the Faculty Board is composed by teachers from the University of Siena: Antonio Acquaviva, Alfio Andronico, Monica Bianchini, Alessandro Cappelli, Anton Ferdinando Carli, Maddalena Cioni, Giuseppe Pasquale Cito, Serenella Civitelli, Antonio De Martino, Paolo Frezzotti, Theodora Hadjistilianou, Marco Lorenzi, Sergio Mancini, Giuseppe Marzocca, Clelia Daniela Anna Miracco, Roberto Ponchietti, Francesco Salvestrini, 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;
- Friburg 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 one can find general information on the School, seminar activities, research projects, and PhD students scientific “identity card”.

Index

Annual progress report day	Pag. 8-9
Students Project Abstracts	Pag. 11-43
• Abbadessa Giovanni <i>Gene Therapy with oncosuppressor genes in Primary Liver Cancer xenograft</i>	Pag. 11
• Ariani Francesca <i>Molecular defect in MECP2-negative Rett patients</i>	Pag. 12
• Artuso Rosangela <i>CDKL5 gene and the early seizure variant of Rett syndrome</i>	Pag. 13
• Barellini Leonardo <i>Prevalence of undetected FAP in patients with papillary thyroid carcinomas</i>	Pag. 14
• Benoni Simona <i>Genotype-Phenotype correlation in Mendelian Syndromes with tumor predisposition</i>	Pag. 15
• Bongini Giada <i>Juvenile colorectal cancer and multiple primitive tumors: genetic involvements</i>	Pag. 16
• Calamati Giulia <i>Outcome of Minimally Invasive Robotic Pancreatectomy</i>	Pag. 17
• Caselli Rossella <i>2q24-q31 deletion syndrome</i>	Pag. 18
• Causarano Vincenza <i>Genetic basis of Premature Ovarian Failure</i>	Pag. 19
• Chessa Antonella <i>Quality of life in colorectal cancer patients after surgery</i>	Pag. 20
• Cisternino Filomena <i>Primary liver tumors in patients with inherited multitumoral syndrome</i>	Pag. 21
• De Robertis Alessandra <i>Pim-1 activates transcription by recruiting chromatin remodeling factors</i>	Pag. 22
• Dhamo Armand <i>Extracolonic manifestation of adenomatous polyposis of the colon</i>	Pag. 23
• Ferrari Eleonora <i>Different roles of Cdk9 isoforms in muscle differentiation</i>	Pag. 24
• Grassi Irene <i>Familial risk in colorectal cancer</i>	Pag. 25
• Guarnaccia Valeria <i>Establishing FAFL4 KO mouse</i>	Pag. 26
• Khadang Baharak <i>X-Linked Alport Syndrome</i>	Pag. 27
• Katzaki Eleni <i>Cohen syndrome: a possible founder effect in North-Eastern Italy</i>	Pag. 28
• Malagnino Giuliana <i>Familial adenomatous polyposis</i>	Pag. 29

• Mario Mancino <i>Cripto 1 expression and tumorigenesis</i>	Pag. 30
• Marcocci Elena <i>Autosomal Alport syndrome</i>	Pag. 31
• Mariani Federico <i>Hypermethylation of multiple genes in pancreatic tumors</i>	Pag. 32
• Ottimo Federica <i>Rett syndrome: MECP2 genotype-phenotype correlation</i>	Pag. 33
• Papa Filomena Tiziana <i>WDR36 is not a glaucoma gene</i>	Pag. 34
• Rizzolio Flavio <i>Defining the role of pRB2/p130-E2F4/5, STAT3 and ERβ in Ovarian Surface Epithelium (OSE) and Ovarian Carcinoma (OC) by CHIP on chip analysis</i>	Pag. 35
• Roberti Annalisa <i>Characterization of novel sporadic Burkitt lymphoma cell line (GAL1): analysis of cell cycle regulation proteins and pRB2/p130 pathways</i>	Pag. 36
• Rosseto Spanhol Ariele <i>Polycystic Kidney Disease</i>	Pag. 37
• Sampieri Katia <i>Searching for modifier gene in retinoblastoma</i>	Pag. 38
• Scala Elisa <i>Italian Rett Database and biobank</i>	Pag. 39
• Speciale Caterina <i>Searching the mental retardation gene in 13q14 deletion syndrome (Retinoblastoma and MR)</i>	Pag. 40
• Squillaro Tiziana <i>LRRK2 mutation analysis in patients affected by Parkinson's disease</i>	Pag. 41
• Vignoli Marina <i>Role of CDKN2A deletions in predisposition to familial melanoma</i>	Pag. 42
• Vogiatzi Paraskevi <i>Immunohistochemical analysis of pRB2/p130, VEGF, EZH2, p53, p16INK4A, p27KIP1, p21WAF1, Ki-67 expression patterns in gastric cancer</i>	Pag. 43
Thesis discussion days	Pag. 44-46

Annual Progress Report Oncology and Genetics Doctoral School October 6, 2006 - room 3 Centro Didattico S. Maria alle Scotte, 8.45

8.45 Introduction

Alessandra Renieri

9.00 Final report of the XVIII cycle (15 minutes for each one)

Chairman Alessandra Renieri

Ariani Francesca (A. Renieri)

Molecular defect in MECP2-negative Rett patients

Bernini Andrea (F. Cetta)

Circulating Gastrin and Ghrelin levels in patients with colorectal cancer: correlation with tumor stage, helicobacter pylori infection and BMI

De Robertis Alessandra (S. Oliviero)

Pim-1 activates transcription by recruiting chromatin remodeling factors

Vogiatzi Paraskevi (A. Giordano)

Immunohistochemical analysis of pRB2/p130, VEGF, EZH2, p53, p16INK4A, p27KIP1, p21WAF1, Ki-67 expression patterns in gastric cancer

10.30 Progress report of the XIX and XX cycles (10 minutes for each one)

Chairman Francesco Cetta

3rd year

Caselli Rossella (A. Renieri)

2q24-q31 deletion syndrome

Dhamo Armand (F. Cetta)

Extracolonic manifestation of adenomatous polyposis of the colon

Mariani Federico (F. Cetta - E. Pinto)

Hypermethylation of multiple genes in pancreatic tumors

Scala Elisa (A. Renieri)

Italian Rett Database and biobank

2nd year

Barellini Leonardo (F. Cetta)

Prevalence of undetected FAP in patients with papillary thyroid carcinomas

Calamati Giulia (F. Cetta)

Outcome of Minimally Invasive Robotic Pancreatectomy

Causarno Vincenza (A. Renieri - D. Toniolo)

Genetic basis of Premature Ovarian Failure

Roberti Annalisa (A. Giordano)

Characterization of novel sporadic Burkitt lymphoma cell line (GAL1): analysis of cell cycle regulation proteins and pRB2/p130 pathways

Sampieri Katia (A. Renieri)

Searching for modifier gene in retinoblastoma

Speciale Caterina (A. Renieri)

Searching the mental retardation gene in 13q14 deletion syndrome (Retinoblastoma and MR)

15.00 Progress report of the 1st year, cycle XXI (5 minutes for each one)
Chairman Maurizio Genuardi

- Artuso Rosangela (A. Renieri)
CDKL5 gene and the early seizure variant of Rett syndrome
- Chessa Antonella (G. Tanzini)
Quality of life in colorectal cancer patients after surgery
- Grassi Irene (G. Tanzini)
Familiar risk in colorectal cancer
- Katzaki Eleni (A. Renieri)
Cohen syndrome: a possible founder effect in North-Eastern Italy
- Malagnino Giuliana (F. Cetta)
Familial adenomatous polyposis
- Mancino Mario (A. Giordano)
Cripto 1 expression and tumorigenesis
- Ottimo Federica (A. Renieri)
Rett syndrome: MECP2 genotype-phenotype correlation
- Squillaro Tiziana (A. Renieri)
LRRK2 mutation analysis in patients affected by Parkinson's disease
- Tirone Andrea (F. Cetta)
- Vignoli Marina (M. Genuardi)
Role of CDKN2A deletions in predisposition to familial melanoma

16.30 Presentation of the PhD students program of the XXII cycle (3 minutes for each one)
Chairman Gabriello Tanzini

- Abbadessa Giovanni (A. Giordano)
Gene Therapy with oncosuppressor genes in Primary Liver Cancer xenograft
- Benoni Simona (F. Cetta - M. De Marchi)
Genotype-Phenotype correlation in Mendelian Syndromes with tumor predisposition
- Bongini Giada (G. Tanzini)
Juvenile colorectal cancer and multiple primitive tumors: genetic involvements
- Cisternino Filomena (F. Cetta)
Primary liver tumors in patients with inherited multitumoral syndromes
- Ferrari Eleonora (A. Giordano)
Different roles of Cdk9 isoforms in muscle differentiation
- Giachino Daniela (A. Renieri - M. De Marchi)
SHOX mutations and pharmacogenetics of rGH in a population of children with short stature
- Guarnaccia Valeria (A. Renieri - P. D'Adamo)
Establishing FAFL4 KO mouse
- Khadang Baharak (A. Renieri)
X-linked Alport syndrome
- Marcocci Elena (A. Renieri)
Autosomal Alport syndrome
- Papa Filomena (A. Renieri)
WDR36 is not a glaucoma gene
- Rosseto Ariele (A. Renieri)
Polycystic kidney disease
- Rizzolio Flavio (A. Giordano)
Defining the role of pRB2/p130-E2F4/5, STAT3 and ER β in Ovarian Surface Epithelium (OSE) and Ovarian Carcinoma (OC) by ChIP on chip analysis

18.30 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.

Oncology and Genetics Doctoral School
Oncological Genetics
XXII cycle
Giovanni Abbadessa, MD
Tutor A. Giordano



Gene Therapy with oncosuppressor genes in Primary Liver Cancer xenograft

Medical therapy for hepatocellular carcinoma (HCC) is currently inefficient. Clinical trials are ongoing to test the efficacy of new molecules, but definitive results are not available yet. Rb2 monitors cell cycle progression mostly by interaction with HCC g with E2F family members of transcription factors and with D cyclins. Its expression levels correlate inversely prognosis and VEGF production. Since angiogenesis is fundamental for the development of this particular kind of cancer, tumor transfection with Rb2 should stop the tumor growth and possibly its related angiogenesis. The addition of other oncosuppressor genes working on different pathways may even cause tumor regression.

METHODS: 2.5×10^6 of Hep3b human liver carcinoma cells will be injected subcutaneously in the flank of nude mice. When the tumors reach a volume of approximately 200 mm³, 5×10^{12} particles of adenoviruses containing pRb2/p130 will be injected intratumorally, in a first group of mice. The same will be done with other oncosuppressor genes, with the combination of these genes, and with GFP controls, in different groups of mice. Each group will be formed by 8 animals, and the experiment will be repeated twice. Results will be evaluated by measuring tumor volumes and weight, by pathological assessment, by dosing the expression of the gene products, of markers of angiogenesis and of cyclins.



Doctorate in Medical Genetics
XVIII cycle
Francesca Ariani, BS
Tutor A. Renieri

Molecular defect in MECP2-negative Rett patients

Rett syndrome (RTT, MIM# 312750) is a devastating neurodevelopmental disorder that represents one of the most common genetic causes of mental retardation in girls. MECP2 mutations account for about 80% of classic RTT cases and for a lower percentage of variant patients. This study has been focused on the identification of the genetic defect in RTT patients without an apparent MECP2 mutation. To this aim, we employed a combination of different strategies including the set up and use of a clinically and molecularly well-defined patients database, the improvement of traditional methods for the analysis of MECP2 and the identification of new genes involved in RTT. By this approach, we identified the molecular cause in 14/74 (19%) mutation-negative RTT patients.

We first searched for MECP2 large rearrangements, not detectable by PCR-based traditional techniques. Using Real Time qPCR, we identified MECP2 large deletions in 9 sporadic classic cases and in one familial case with two RTT sisters, one "highly functioning" PSV and the other with classic RTT. These results indicate that MECP2 large deletions are an important cause of classic RTT and confirm the importance of quantitative studies in a complete diagnostic strategy. Subsequent analysis by MLPA showed that all rearrangements are partial MECP2 deletions, involving only exon 3 and 4 of the gene. We next searched for mutations in exon 1, previously considered non coding and therefore excluded from MECP2 mutation analysis. By DHPLC, we identified a deletion in one classic patient, suggesting that exon 1 mutations are not common in RTT.

Among atypical RTT cases, we also identified mutations in another gene, CDKL5, demonstrating the genetic heterogeneity of the syndrome. In particular, we found CDKL5 frameshift mutations in 4 RTT patients, all with the infantile seizure onset variant of RTT. Considering the similar phenotypes caused by mutations in MECP2 and CDKL5, we hypothesized that the two genes play a role in common pathogenic processes. We demonstrated that CDKL5 is a nuclear protein whose expression in the nervous system overlaps that of MeCP2, during neural maturation and synaptogenesis. We also demonstrated that MeCP2 and CDKL5 interact both in vivo and in vitro and that CDKL5 is able to phosphorylate itself and to mediate MeCP2 phosphorylation. These results support the hypothesis that the functions of MECP2 and CDKL5 are tightly linked and contribute to explain the involvement of CDKL5 in RTT. Additional studies are necessary to define this new molecular pathway, other components of which could be responsible for the remaining percentage of mutation-negative RTT cases.

Part of this work is published in: Mari F et al. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. *Hum Mol Genet.* 2005 Jul 15;14(14):1935-46.

Scala E. Ariani F. et al. CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. *J Med Genet.* 2005 Feb;42(2):103-7.

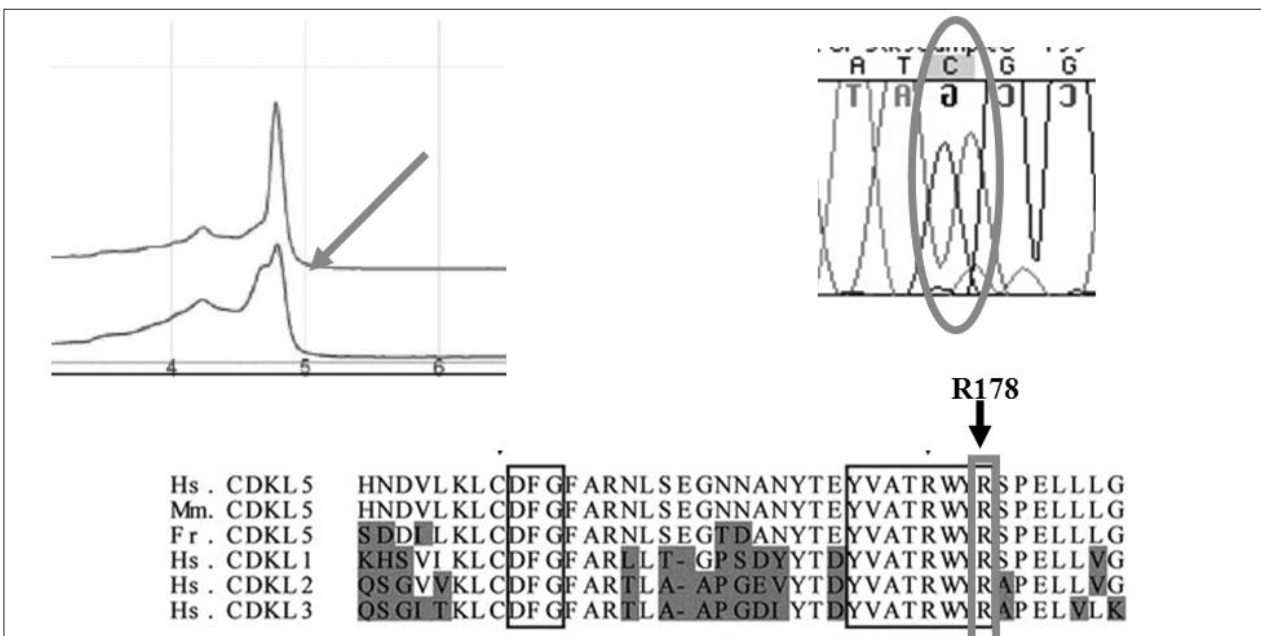
Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
Rosangela Artuso, BS
 Tutor A. Renieri



CDKL5 gene and the early seizure variant of Rett Syndrome

Rett syndrome (RTT, OMIM#312750) is a severe neurodevelopmental disorder almost exclusively affecting females and characterized by a wide spectrum of clinical manifestations. Besides the classical form, several RTT variants have been described. Mutations in the MECP2 gene are found in 80% of classic RTT cases and 20-40% of variants patients. In particular, in the early onset seizures variant of RTT, MECP2 mutations have never been published. In this variant, firstly described by Hanefeld in 1985, the normal perinatal period is soon followed by the appearance of seizures, usually infantile spasms. In 2005, we identified mutations in the CDKL5 gene in four patients with early onset seizures variant, demonstrating genetic heterogeneity of RTT. Subsequently, we found that CDKL5 belongs to the molecular pathway of MeCP2.

Here, we report the identification of another early onset seizures variant patient with a CDKL5 mutation (c.532C>T, p.R178W). The substitution, located in exon 8, has not been found in parents' DNA. The mutated amino acid, Arg178, lies within kinase subdomain VIII which is important for substrate recognition. Exchange of a positively charged Arginine for an uncharged Tryptophan would like influence substrate-binding specificity. Moreover, the mutated amino acid is conserved in mouse and Fugu rubripes Cdkl5 orthologs and is also present in other closely related protein kinases, including CDKL1, CDKL2 and CDKL3. Taken together, these results indicate the pathogenic nature of this CDKL5 missense mutation, never reported before.





Surgery Doctoral School
Hepatobiliopancreatic Disease and Multitumoral Syndromes
XX cycle
Leonardo Barellini, MD
Tutor F. Cetta

Prevalence of undetected FAP in patients with papillary thyroid carcinomas

Familial adenomatous polyposis (FAP) is an autosomal inherited syndrome characterized by the presence of hundreds of adenomatous colonic polyps occurring at an early age. Thyroid carcinoma is one of the several extracolonic diseases that can be discovered in this syndrome. On the basis of restriction criteria the aim of this study will be to find additional patients with FAP in a population of subjects affected by thyroid cancer in order to report the prevalence of the syndrome and to assess whether some screening methods (beta-catenin immunohistochemical study, genetic analysis) may be reliable and cost-effective. Between 2000 and 2005, 270 patients were selected and included in the study. All of these were less than 30 years old and were submitted to a total thyroidectomy after a diagnosis of papillary thyroid carcinoma was achieved. There were 68 men (24.2%) and 202 women, the mean age was 23 years. Of 270, 100 envelopes came back. None of the patients was affected by any of the diseases reported in the questions. Only in three cases (3%) relatives were affected by intestinal polyps, in six cases (6.3%) relatives were diagnosed as having a colon cancer, and in one case a relative died because of that tumour. We had 5 diagnosis of CHPRE (4.8%) in relatives of our patients. We also had 2 (2.1%) gastric cancers, 5 breast cancers (5.2%), one pancreatic cancer (1%) and 2 (2.1%) liver cancers in the kindred. The specimens of CHPRE positive patients' relatives were reviewed looking for the cribriform and morular histotype, which has been shown to be strictly related to thyroid carcinomas associated to FAP syndrome. Patients with relatives affected by CHPRE have been proposed in continuing the study by a pedigree and a genetic analyses, furthermore the specimens has been evaluated for immunohistochemical research of beta-catenins.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Diseases and Multitumoral Syndromes
XXII cycle
Simona Benoni, BS
Tutor F. Cetta - Co-tutor M. De Marchi



Genotype-Phenotype correlation in Mendelian Syndromes with tumor predisposition

The first phase of this project is focused on the PTEN gene.

PTEN (Phosphatase, TEnsin homologue, deleted on chromosome TEN, 10q23.3) encodes a lipid- and protein phosphatase which signals down the phosphoinositol-3-kinase/Akt pathway and determines G1 cell cycle arrest and apoptosis. Alterations of this protein may result in over-proliferation of cells, resulting first in hamartomatous growth and secondary in the possibility of tumor onset; therefore PTEN is now considered as a critical tumor suppressor.

Germline PTEN mutations have been found to occur in 85% of classic Cowden syndrome (CS), 65% of Bannayan-Riley-Ruvalcaba syndrome (BRRS), up to 20% of Proteus syndrome (PS), and approximately 50% of a Proteus-like syndrome (PSL).

Cowden syndrome is a multiple hamartoma syndrome, with autosomal dominant inheritance which usually manifests by the age of 20 years. Characteristic signs are mucocutaneous lesions, thyroid abnormalities, fibrocystic disease, macrocephaly, intestinal polyps and a high risk of breast, thyroid, uterine leiomyoma and endometrial carcinomas.

BRRS is a congenital autosomal dominant disorder characterized by macrocephaly, developmental delay, lipomatosis, and speckled penis. Hamartomatous polyps of the gastrointestinal tract occur in 40% of the patients.

At beginning, CS and BRRS were considered to be as two distinct diseases, but germline mutations of PTEN have been found to be responsible for both.

In fact, PTEN mutation analysis in CS and BRRS patients reveals that 65% of CS-associated mutations occur in the first five exons encoding the phosphatase domain and the promoter region, while 60% of BRRS-associated mutations occur in the four exons encoding mainly the 3'-C2 domain.



Oncology and Genetic Doctoral School
Colorectal and Gastroesophageal Diseases
XXII cycle
Giada Bongini, MD
Tutor G. Tanzini

Juvenile colorectal cancer and multiple primitive tumors: genetic involvements

Colorectal cancer is usually diagnosed in the 6-7th decade, but about 10% of cases arises in younger people.

Early onset is one of the characteristics of hereditary forms, in addition at the occurrence of multiple primary tumours in the same patient, both in the colon and in extracolonic sites.

A clear family history could be seen in about 60-70 % of ascertained hereditary form: in the other cases, a new-onset germline mutation should be found in the proband and he will be the progenitor of a vertical transmission.

The aim of our study is to analyze the incidence of these characteristics in a cohort of patients treated for colorectal cancer at our Institution in the last 20 years.

Pedigrees of patients with such characteristics will be traced.

Paraffin-embedded specimens of multiple primary tumours and of early-onset colorectal cancer will be analyzed for MSI: patients with tumours MSI+ will be invited to give a blood sample to test DNA for a mutation in the MMR genes.

Doctorate in Hepatobiliopancreatic Surgery
and Multitumoral Syndromes
XIX cycle
Giulia Calamati, MD
Tutor F.Cetta



Outcome of Minimally Invasive Robotic Pancreatectomy

Ductal adenocarcinoma is the most common primary malignancy of the pancreas and usually is diagnosed at a late state despite progress in diagnostic imaging, identification of tumour markers and advantage in molecular biology. The minority of patients (10-15%) have a resectable cancer. Surgical resection provides the only chance for cure or long-term survival (overall 5 years survival rate 13%). The most important factors associated with a favourable prognosis include: age, cancer free surgical margins, negative lymph node status, small tumour size, well/moderate tumour differentiation, low perioperative blood loss, tumour location, molecular genetic findings. Relationship between the occult metastasis and micrometastasis, detected using immunohistochemistry and molecular biological studies, and survival remains controversial. Recently, the role of laparoscopy in pancreatic surgery has gained interest and currently has many applications in treatment of benign and malignant lesions by highly skilled laparoscopic surgeons with good results in terms of postoperative recovery, complications, and oncological clearance. Laparoscopic pancreaticoduodenectomy(PD) is still controversial procedure because technical difficulties in the reconstruction stage. With the advent of robotic abdominal surgery the technical limitations of laparoscopy have been overcome. In our data we report 66 cases of pancreatic robotic resections. In 35/66 cases we have performed a PD (10 Longmire + 25 Whipple), in 26/66 cases a distal pancreatectomy (DP) (12 spleen preserving), in 3/66 cases a middle segment pancreatectomy and 2/66 cases an insulinoma enucleation. 14 cases (10 PD and 4 DP), 9 women and 5 men with a mean age of 60,10 years, were performed for adenocarcinoma. We conducted a retrospective analysis to assess surgical outcome and short term follow-up results at 6,12,24,36,38 months and we propose a perspective study to value the factors who influencing long survival. Minimally invasive robotic pancreatectomy for malignancy seem to represent a good alternative to open surgery but longer follow-up periods are needed to establish the current results.



Doctorate in Medical Genetics
 XIX cycle
Rossella Caselli, BS
 Tutor A. Renieri

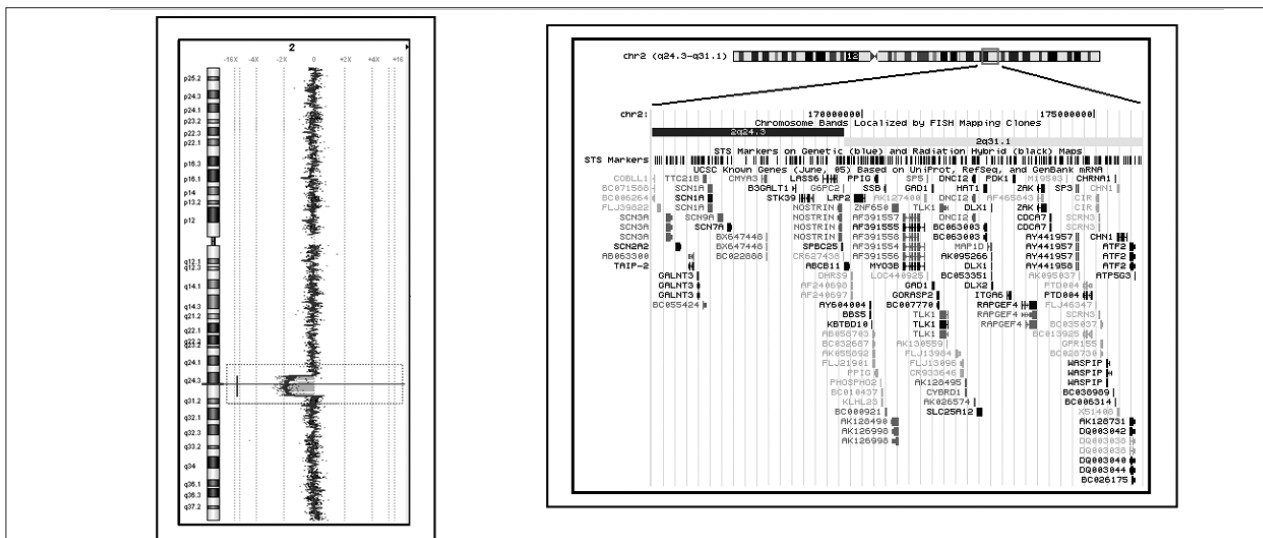
2q24-q31 deletion syndrome

We report here the identification of a de novo interstitial deletion of the long arm of chromosome 2 involving segment between cytogenetic bands 2q24.3 and 2q31.1. To date, more than 100 cases with deletions of the long arm of chromosome 2 have been identified through standard cytogenetic analysis. The most frequent 2q deletion involves the cytogenetic bands 2q31-q33 and corresponds to a specific phenotype. Deletions involving different segments of the long arm of chromosome 2 are characterized by variability in breakpoints location and are clinically heterogeneous.

The patient shows postnatal growth retardation, microcephaly, down-slanting palpebral fissures, long eyelashes and micrognathia. Halluces are long, broad and medially deviated, while the other toes are laterally deviated and remarkably short with hypoplastic phalanges. She also showed developmental delay, seizures, lack of eye contact, stereotypic and repetitive hand movements and sleep disturbances. In order to establish the associated phenotype, we have attempted to compare the clinical features of our case with the phenotypes of 12 reported patients with overlapping deletions. The emerging phenotype includes low birth weight, postnatal growth retardation, mental retardation and developmental delay, microcephaly, and peculiar facial dysmorphisms.

We performed gene content analysis of the deleted region to investigate the presence of genes that may be good candidates in generating both neurological and dysmorphic phenotype of the patient. The gene content analysis of the deleted region shows the presence of 58 known genes. Among them, DLX1 and DLX2 genes are of particular interest due to their role in control craniofacial patterning and differentiation and survival of forebrain inhibitory neurons. Other good candidate genes located within the deleted region are SCNA genes. It is known that loss of function mutations in this gene cause a severe form of epilepsy.

Taken together, these results indicate to accurately evaluate the 2q24-q31 region in that cases presenting a syndromic condition with mental retardation, developmental delay, severe epilepsy and digital anomalies.



Pescucci C et al., 2q24-q31 deletion: report of a case and review of the literature Eur J Med Genet, 2007Jan-Feb; 50(1):21-32

Medical Genetics Doctoral School
 Medical Genetics
 XX cycle
Vincenza Causarano, BS
 Tutor: A. Renieri
 Co-tutor D. Toniolo



Genetic basis of Premature Ovarian Failure

Premature Ovarian Failure (POF) is a disorder of ovulation characterized by primary or secondary amenorrhea (before 40 years of age), or early menopause (before 45 years of age) and elevated serum gonadotropins level.

Known causes of POF include environmental factors, such as radiation, chemotherapy and infections. A genetic component of the disorder is demonstrated by cases associated with X chromosome rearrangements and by a significant fraction of familial cases. Also POF is thought to be due to the contribution of several genes acting as risk factors.

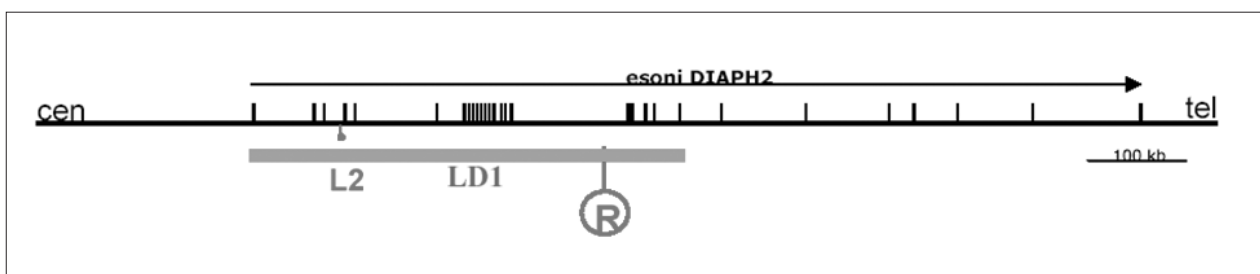
DIAPH2 is one of the three human homologues of the *Drosophila melanogaster* dia gene. In the fruit fly, the gene is responsible for defects in oogenesis and spermatogenesis and causes sterility in both sexes. The DIAPH2 gene was found interrupted, in a POF familial case, by the breakpoint of balanced X;12 translocation. Mutation analysis on a large cohort of Italian patients failed to demonstrate any causative variants.

A large region of DIAPH2 gene is contained in a linkage disequilibrium block where an intronic variant (L2), 25 bp upstream exon 4, was associated with POF. We searched for a functional variant in the linkage disequilibrium block that may represent the risk factor for POF: we looked for this variant in 50 bp upstream and downstream from splice sites, in peri-exonic and intronic conserved sequences and in promoter region.

Analysing these regions, at about 4 kb upstream transcription start site, we found a variant, RS18, associated with POF.

Due to the position of RS18 variant, we investigated if the region containing the SNP has a promoter or enhancer function. We cloned a 1 kb region containing RS18 variant into luciferase expression vectors and we transfected into HeLa cells. We found that this region has no promoter activity and has an enhancer effect. Now we are studying if the RS18 alleles have different effects on gene expression.

In addition, since the region from exon 1 to exon 6 is involved in alternative splicing, we are studying if one of the alleles of L2 variant can affect the regulation of splicing.





Oncology and Genetics Doctoral School
Colorectal and Gastroesophageal Disease
XXI cycle
Antonella Chessa, MD
Tutor G. Tanzini

Quality of life in colorectal cancer patients after surgery

An evaluation of quality of life has been conducted by the use of specific questionnaires -European Organization for Research and Treatment of Cancer QLQ C30 QLQ CR38 modules-during the standard program of clinical and instrumental follow up for colorectal cancer patients.

In the present study all rectal cancer patients operated between November 2005 and September 2006 have been enrolled. Four out of ten subjects have completed the questionnaires. Functional scales and symptom items have been scored at 3,6,9 and 12 months. All scales range from 0 to 100 with a higher scale score representing a higher level of functioning or symptomatology.

Preliminary scores suggest that rectal cancer surgery has specific variables involved with quality of life as patient age, radiotherapy, surgical technique in relation with local cancer diffusion and need of a colostomy. Age in male subjects influences functional outcome concerning sexual items: patients under 65 years have a more efficient communication about these problems and appears more interested in solving them. Pelvic floor stimulation may have a role in functional items.

Further data are under collection.

This survey can drive to a better multidisciplinary management in order to improve the short and middle time results in terms of quality of life not only as disease free survival rates but also as overall wellbeing.

Oncology and Genetics Doctoral School
Hepatobiliopancreatic Disease and Multitumoral Syndromes
XXII cycle
Filomena Cisternino, BS
Tutor F. Cetta



Primary liver tumors in patients with inherited multitumoral syndromes

Primary liver tumors may occur as part of some inherited multitumoral syndromes. In particular hepatoblastoma (HB) is a well known manifestation of familial adenomatous polyposis (FAP) and of Beckwith-Wiedemann syndrome (BWS).

Familial adenomatous polyposis (FAP) is an inherited multitumoral syndrome, with affected patients showing hundreds to thousands of adenomatous colonic polyps, invariably evolving into carcinomas between 30 and 50 years of age. In addition to colonic tumors, desmoids, osteomas, thyroid carcinoma, hepatoblastoma (HB) and, less frequently, other primary liver tumors may occur.

The genetic defect is a germ-line mutation of the APC gene, mapped at chromosome 5q21.

HB is an embryonic tumor usually consisting of an epithelial and mesenchymal component, which mainly occurs in the first years of life. We have observed 2 patients with FAP associated primary livers tumors and we have collected 5 addition patients, thanks to an international cooperation. In addition, we analyzed data and specimens from 2 patients with BWS and HB.

Aims of the research:

- 1) studies of genotype-phenotype correlation;
- 2) genetic analysis of germ-line or somatic mutation of APC;
- 3) study of beta-catenin overexpression and/or mutation of the beta-catenin gene.

APC and beta-catenin are part of the WNT-Wingless pathway. Beta-catenin mutation and its nuclear overexpression seems to be involved in tumorigenesis and cell differentiation. In addition, to analyze patients with known inherited syndromes we also studied 20 sporadic HBs and 11 non virus-related HCCs. In the HB group the mean age was 1 year and 7 months whereas in the HCC group all the patients were less than 40-years-old and the tumor had developed in the absence of cirrhosis. Mutational analysis of a part of the exon 3 of the beta-catenin gene as well as the research of microsatellite instability were performed in all HBs and HCCs. SSCP analysis

Sequencing of genomic DNA showed a mutation of the beta-catenin gene in 2 HBs (10%) and 2 HCCs (18%). A missense mutation in the codons 32 and 38 was discovered in 2 HBs while a nonsense mutation in the codon 27 and a missense mutation in the codon 32 were found in 2 HCCs. Microsatellite instability was also found in 11 HBs. Our preliminary data seem to confirm beta-catenin involvement in liver carcinogenesis, and, interestingly, suggest that a high percentage (55%) of microsatellite instability occurs in HBs.



Doctorate in Medical Genetics
XVIII cycle
Alessandra De Robertis, BS
Tutor S. Oliviero

Pim-1 activates transcription by recruiting chromatin remodeling factors

The serine/threonine kinase Pim-1 is involved in cell cycle progression and differentiation.

Here we show that Pim-1 contributes to the activation of Myc-target genes by phosphorylating the nucleosome at Serine 10 (S10) of histone H3. Following growth factor stimulation Pim-1 is recruited by Myc to the chromatin at the FOSL1 enhancer, to the E boxes of early genes and RNAi experiments show that Pim-1 is required for H3S10 phosphorylation and transcriptional activation of these genes.

Moreover, we demonstrate that a MAX/MYC/PIM-1 nuclear complex is able to bind the FOSL1 enhancer and at E boxes of other genes and to phosphorylate H3 in a Pim-1-dependent manner.

These results establish a new function of Pim-1 as a chromatin modifier at Myc-target loci and suggest that nucleosome phosphorylation, at E boxes, is required for triggering transcriptional activation.

Doctorate in Hepatobiliopancreatic Surgery and Multitumoral Syndromes
XIX cycle
Armand Dhamo, MD
Tutor F. Cetta



Extracolonic manifestation of adenomatous polyposis of the colon

Recently was reported a patient with the cribriform-morular variant of papillary thyroid carcinoma (CMV/PTC), associated with familial adenomatous polyposis (FAP) (Uchino et al. World. J. Surg. 2006). The molecular analysis of APC gene and the b-catenin gene in the peripheral blood and in 12 CMVPTC nodules was performed. A germ-line mutation of the APC and six somatic mutations were found, there were no mutations of the bcatenin gene either in peripheral blood leukocytes or in the 12 CMVPTC specimens. In particular, loss of heterozygosity was not observed in the tumor tissues without somatic APC mutations. Previous data suggest that APC mutations and b-catenin mutations are mutually exclusive of each other in disrupting the Wnt signal transduction; in thyroid carcinoma as well as in hepatoblastoma, b-catenin mutations usually occur in the absence of APC mutations and viceversa. In addition, 1) somatic mutations of the APC gene are very rare in FAP associated thyroid carcinoma, and in sporadic thyroid carcinoma 2) LOH of the APC gene is extremely rare even in FAP associated thyroid carcinoma. We found no LOH for APC in 6 of 6 patients with FAP associated PTC.

In conclusion, cumulative data, associated with 1) the low incidence of PTC in FAP patients (< 2%), 2) the almost exclusive occurrence in females (F:M ratio > 17:1), a very high rate of RET/PTC activation (> 50%), similar to that observed in post Chernobyl children, suggest that mechanisms other than biallelic loss of function of the APC gene, which is found only in a minority of patients, are responsible for the occurrence of most FAP associated PTCs.

we suggest that in most of cases APC germline mutations only give a generic susceptibility to thyroid cancer development, whereas other concomitant factors (sex related factors, modifier genes, environmental factors, namely exposure to radiation or even long distance nuclear disasters) all play a role in the actual occurrence of PTC in FAP patients.

Part of this work was published in:

Cetta et al., Germ-line and Somatic Mutations of the APC Gene and/or β Catenin Gene in the Occurrence of FAP Associated Thyroid Carcinoma World J Surg (2007) 31:1366–1367



Oncology and Genetics Doctoral School
Oncological Genetics
XXII cycle
Eleonora Ferrari, DVM
Tutor A. Giordano

Different roles of Cdk9 isoforms in muscle differentiation

Cdk9 is a Cdc2-like kinase whose expression, unlike the other Cyclin-dependent kinases (CDKs), is not serum- or cell cycle-regulated. Cdk9 regulates several physiological processes, such as transcription, apoptosis and differentiation. Cdk9 and its cyclin partner Cyclin T belong to the Positive Transcription Elongation Factor b (P-TEFb), which is involved in the regulation of transcriptional elongation. Cdk9 activates transcription elongation by phosphorylating the carboxyl-terminal domain (CTD) of RNA polymerase II.

Cdk9 is ubiquitously expressed, but its expression is higher in terminally differentiated tissues, suggesting for this kinase an active role in differentiation. We have previously demonstrated that Cdk9 is involved in neural differentiation, B cell activation/differentiation and in myogenesis. During muscle differentiation Cdk9 is up-regulated and is associated with Cyclin T2a (Cyc T2a). The Cdk9/Cyc T2a complex binds to and phosphorylates MyoD. This strengthens MyoD-dependent transcription and stimulates myogenic differentiation in both MyoD-converted fibroblast and C2C12 mouse muscle cells.

In contrast, inhibition of Cdk9 activity by a dominant negative form (Cdk9-dn) represses the myogenic program.

Recently, two different isoforms of Cdk9 have been described. The aim of this project is to evaluate whether they can have different roles. In particular we aim to investigate which isoform is specifically activated during muscle differentiation. The understanding of the biological processes underlying muscle differentiation and regeneration may be of help for the understanding of neurodegenerative disorders and myopathies.

Oncology and Genetic Doctoral School
Colorectal and Gastroesophageal Diseases
XXI cycle
Irene Grassi, MD
Tutor G.Tanzini



Familial risk in colorectal cancer

Colorectal cancer is the second leading cause of death for neoplastic diseases in Western World and its incidence is increasing.

Cumulative cancer-related deaths are high (about 50%), mostly because a large amount of cancers are diagnosed in advanced stages.

Prevention and early diagnosis are the most useful tools for abating these mortality rates since the large bowel cancerogenesis is an evolving process (so-called adenoma-carcinoma sequence) that may be interrupted by removing the precancerous lesions.

Familiarity is a well-known risk factor both for adenomas and cancer and can be used to optimize cost-benefit programs of screening.

Furthermore, the familial risk may be more elevated if potential-related characteristics of hereditary colorectal cancer are present (juvenile age of onset, multiple primary tumours, several affected in the same family, histologic and molecular features..).

All these characteristics have been evaluated in a consecutive cohort of patients operated for colorectal cancer at our Institution. The following are the preliminary results.

From 2005 to september 2006, we traced the pedigrees of 175 patients: at least one first-degree relative with the same cancer was reported by 29 of them (16,11 %).

In 5 patients a hereditary form could be hypothesized and a DNA testing has been offered.

A FAP was ascertained in a family (APC mutation positive).

In another family an attenuated form (AAPC) was suspected and biomolecular analysis is in progress.



Oncology and Genetics Doctoral School
Medical Genetics
XXII cycle
Valeria Guarnaccia, BS
Tutor A. Renieri - Co-tutor P. D'Adamo

Establishing FA CL4 KO mouse

Mental retardation is a condition characterized by significantly below average intellectual functioning (IQ<70) in conjunction with significant limitations in adaptive functioning, occurring before the age of 18 years. An excess of males in mentally retarded patients has long been noticed and explained by the presence of mutations in genes on the X chromosome. It is calculated that X linked mental retardation (XLMR) may account for about 20-25% of mentally retarded males. Usually, XLMR is divided into "syndromic" (MRXS) and "non-specific" (MRX) forms, depending on the presence or absence, besides mental retardation, of a specific pattern of physical, neurological, or metabolic abnormalities

Piccini et al. (1998) reported that the 2 patients with Alport syndrome, elliptocytosis, and mental retardation described by Jonsson et al. (1998) carried a large deletion of the COL4A5 region that included the contiguous FA CL4 gene. It suggested that the absence of FA CL4 might play a role in the development of mental retardation or other signs associated with Alport syndrome in these patients.

Long chain acyl-CoA synthetase (LACS), or long chain fatty acid-CoA ligase (FA CL), converts free long chain fatty acids into fatty acyl-CoA esters, which are key intermediates in the synthesis of complex lipids. FA CL4 gene contains 16 exons and spans approximately 90 kb and encodes a form of LACS and is expressed in several tissues, including brain.

In a family with nonspecific X-linked mental retardation (MRX68), Longo et al. (2003) identified a 1001C-T change in the FA CL4 gene, resulting in a pro375-to-leu (P375L) substitution in the affected members. Carrier females had 100% skewed X inactivation. Functional studies of the mutant protein showed a marked reduction in enzyme activity. The authors suggested that reduction of FA CL4 activity may lead to deranged fatty acid metabolism in neurons, causing defects of neuron outgrowth, synaptogenesis, and other developmental functions important for normal brain development. For this reason we think that the study of the molecular mechanisms causing human MR requires the use of animal models and we suggest that the mouse represent a good model to study the role of human FA CL4 gene in the establishment of cognitive functions and in MR. A FA CL4 KO mouse will be generated and it will be analyzed from a molecular and behavioral point of view. Despite the obvious difference in cognitive functions between mouse and human, it is not unlikely that basic molecular mechanisms common to both species may be altered by mutations in MR genes and that their identification in the mouse will lead to new insights into the human defects.



Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
Eleni Katzaki, BS
 Tutor A. Renieri

Cohen syndrome: a possible founder effect in North-Eastern Italy

Cohen syndrome (OMIM #216550) is an recessively inherited condition firstly described by Cohen et al.(1973) Initially it was characterized in Finish population where the phenotype is highly homogeneous, consisting of a non progressive mild to severe psychomotor retardation, motor clumsiness, microcephaly, characteristic facial features, childhood hypotonia and joint laxity, progressive retinochoroidal dystrophy, myopia, intermittent isolated neutropenia. In this population a hotspot mutation has been described (p.C1117fsX1124), the frequency of which probably results from a founder effect. On the contrary, the different ethnic background patients reported by Seifert et al; demonstrate an obviously broad phenotypic variability .

The CS phenotype was found to be associated with mutations in the gene COH1 in different populations. COH1 maps to chromosome 8q22 and codes for 5 splice forms, it consists in 62 exons and encodes a potential transmembrane protein presumably involved in vesicle mediated sorting and intracellular protein transport.

In my project of research I am performing the molecular analysis of 21 patients using transgenomic WAVE denaturing high performance liquid chromatography (DHPLC). In most families we found private mutations except of a single base deletion (T3708fs3769X) in the COH1 gene found in three apparently unrelated Italian families with Cohen syndrome deriving from a restricted area of the Veneto's lowland (between Padova and Tagliamento).

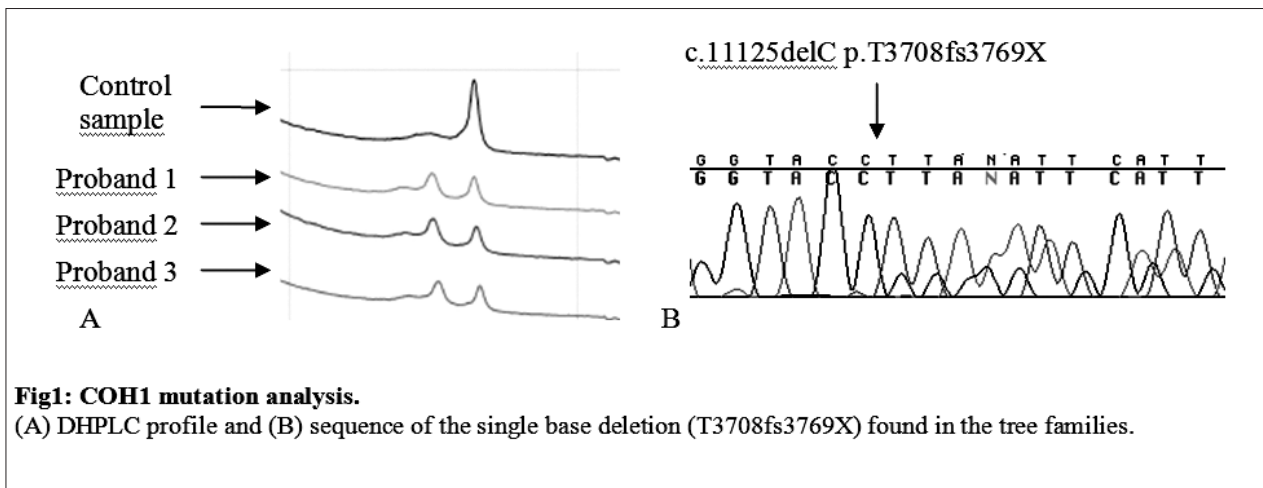


Fig1: COH1 mutation analysis.
 (A) DHPLC profile and (B) sequence of the single base deletion (T3708fs3769X) found in the tree families.

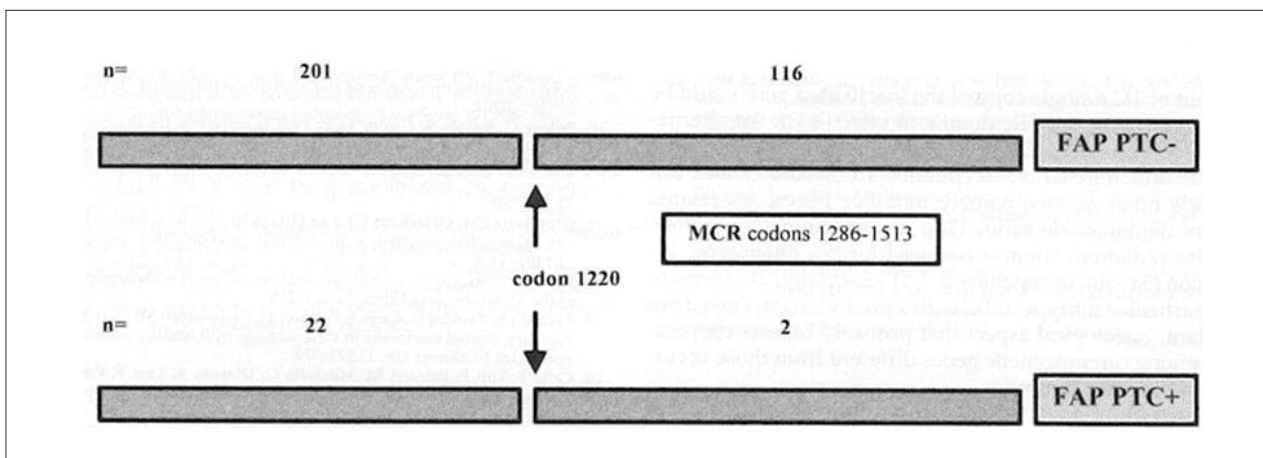
Oncology and Genetics Doctoral School
 Hepatobiliopancreatic Disease and Multitumoral Syndromes
 XXI cycle
Giuliana Malagnino, PCS
 Tutor F. Cetta



Familial adenomatous polyposis

Familial Adenomatous Polyposis (FAP) is a multitumoral syndrome due to germ-line mutation of the APC gene (mapped at 5q21). In addition to hundreds to thousand colonic polyps, which are going to transform invariably into malignant tumors before age 50, the syndrome includes: gastric and duodenal polyps, periamullary tumors, primary liver tumors, carcinoma of thyroid, tumors of the central nervous system and desmoid tumors. In addition, benign alterations such as CHRPE (Congenital Hypertrophy of the Retinal Pigment Epithelium), osteomas and dental abnormalities have been reported. In particular the research will focus on FAP associated papillary thyroid carcinoma. In 22 out of 24 patients with FAP associated papillary thyroid carcinoma (PTC), germ-line mutations of the APC gene clustered before codon 1220 were found. Usually the same genomic area is associated with a non malignant alteration such as congenital hypertrophy of the retinal pigment epithelium (CHRPE). Interestingly, 4 out of 5 of these patients had activation of the ret gene in the thyroid tumor tissue (ret/PTC1 isoform). The aims of the research will be: 1) to Studies the genotype-phenotype correlation, i.e. correlation between the site of APC mutation and occurrence of CHRPE and PTC in different families; 2) To Search for LOH of the APC gene in the peripheral tissue of FAP patients, either malignant (thyroid tumors) or benign (CHRPE), to confirm or exclude the two-hit Knudson hypothesis in the occurrence of these manifestations; 3) To perform Analysis of ret/PTC isoform in different families with FAP associated PTC.

In particular, during the last year, we have collected 4 new patients with CHRPE and thyroid carcinoma in females aged less than 30 years. These patients could be index cases for previously undetected FAP syndromes. In addition, we have collected all reports published in the world literature concerning FAP associated thyroid carcinoma and up-dated the series of patients with this peculiar extracolonic manifestation of FAP. In particular, a strict correlation has been observed between FAP and a very unusual variant of thyroid carcinoma, the so called "cribriform morular variant". Intensive screening for FAP, both clinical and genetic, has been made in these patients and relatives. This could facilitate a better knowledge of genotype-phenotype correlation and a deeper insight into the Wnt-pathway.





Oncology and Genetics Doctoral School
Oncological Genetics
XXI cycle
Mario Mancino, BS
Tutor A. Giordano

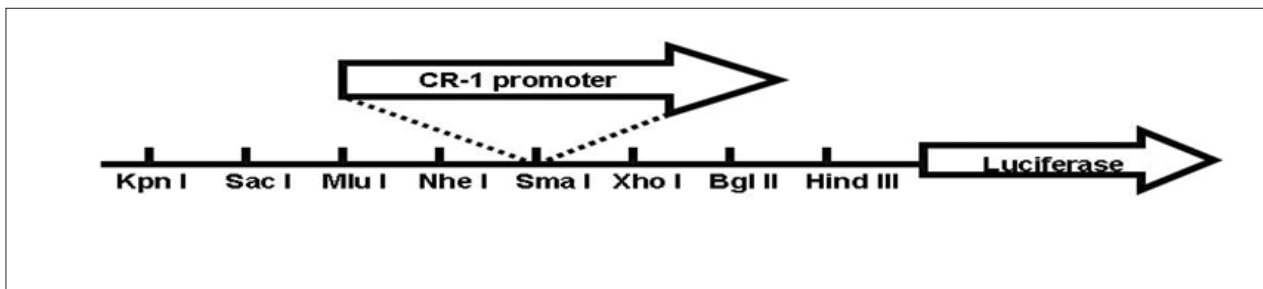
Cripto 1 expression and tumorigenesis

Human Cripto-1 (CR-1), a member of the Epidermal Growth Factor-Cripto-1/FRL1/cryptic (EGF-CFC) family of proteins consists of extracellular and cell-associated proteins identified in several vertebrate species. CR-1 is involved in embryonic development and is overexpressed in a number of human cancers. In fact, CR-1 can induce cell proliferation, survival, migration, invasion and angiogenesis in several mouse and human epithelial cells, suggesting that it may play a role during tumorigenesis. Nothing is known about regulatory mechanisms that affect Cripto expression.

The purpose of this study is to assess effects of the members of the Tgf- beta family of signaling molecules (Activin A, Activin B and BMP-4) and RA in human embryonal carcinoma cell line (NTERA2/D1) on full-length and deletion constructs for activation or inhibition of CR-1 Promoter Activity.

To accomplish this, a luciferase reporter assay was developed where the CR-1 promoter was cloned into a pGL3 enhancer-luciferase reporter vector.

Future work will determine whether this assay can be used for high throughput screening of potential drugs or agents that reduce CR-1 expression.



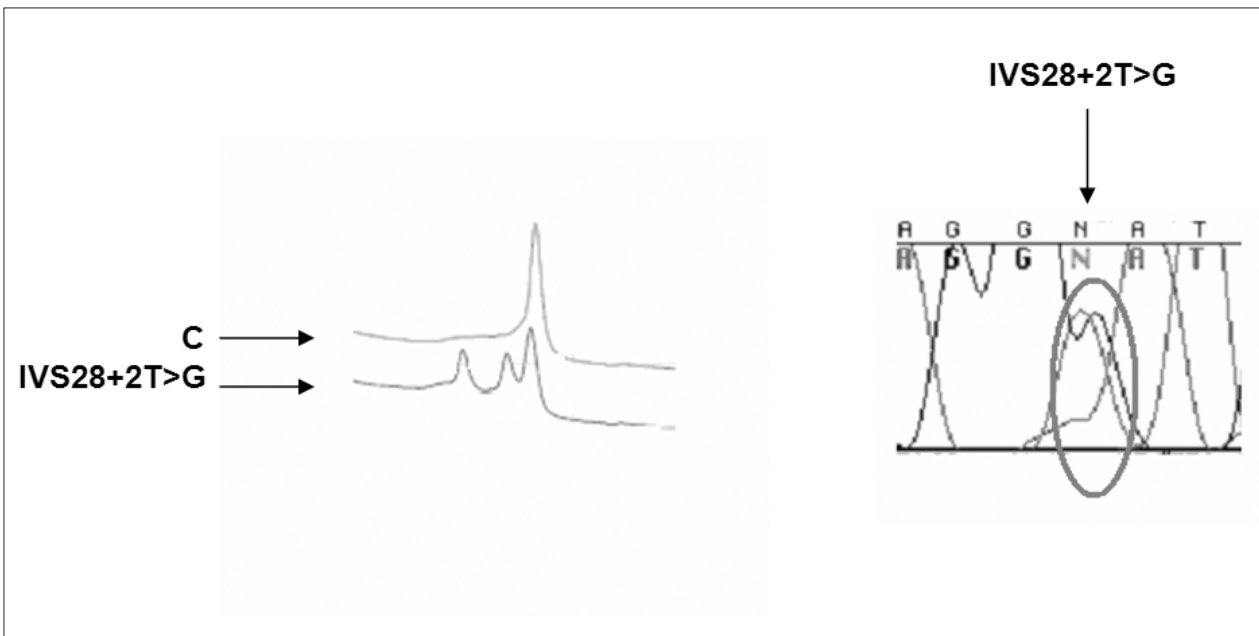
Oncology and Genetics Doctoral School
XXII cycle
Elena Marcocci, BS
Tutor A. Renieri



Autosomal Alport syndrome

Alport syndrome (ATS) is a progressive inherited nephropathy characterized by irregular thinning, thickening and splitting of the glomerular basement membrane often associated with hearing loss and ocular symptoms. The majority of cases (85%) are transmitted as an X-linked semidominant condition due to COL4A5 mutations. However, up to 15% of cases are autosomally inherited and are due to mutations in either COL4A3 or COL4A4 genes. In the autosomal recessive (AR) form both males and females are severely affected. The autosomal dominant (AD) form has been described only recently and its natural history is mostly unknown. A large cohort of autosomal ATS patients will be investigated in order to clarify the natural history of this disorder. Mutation analysis by Denaturing High Performance Liquid Chromatography has been already started and a mutated profile in COL4A4 gene has been revealed. Automated sequencing was performed and allowed us to identify the mutation IVS28+2T>G; its segregation within the family is indicative of an AD-ATS. This is the 6th family affected by the rare AD-ATS form reported by our group.

My research project focus on the analysis of the COL4A4 and COL4A3 genes in a large cohort of patients, using Denaturing High Performance Liquid Chromatography (DHPLC) and automated sequencing. The identification of new families with AD-ATS and AR-ATS can contribute to explain the evolution of this forms of ATS.





Doctorate in Hepatobiliopancreatic Surgery
XIX cycle
Federico Mariani, MD
Tutor F. Cetta - Co-tutor E. Pinto

Hypermethylation of multiple genes in pancreatic tumors

Aberrant DNA hypermethylation in carcinomas usually occurs at CpG islands (regions of DNA rich in CpG dinucleotides). CpG islands are located mainly in 5' regulatory regions of genes. Aberrant promoter methylation of tumor suppressor genes is associated with a loss of gene function. Hypermethylation of CpG islands is a common mechanism by which tumor suppressor genes and mismatch repair genes are inactivated. The identification of genes targeted by hypermethylation may provide insights into tumor-suppressive pathways inactivated in pancreatic cancer. Hypermethylation of promoter regions correlates directly with the loss of transcription of tumor suppressor genes and mismatch repair genes (E-cadherin, hMLH1, P16, TIMP3, RAR- α).

Hypermethylation of these CpG islands did not occur randomly in these carcinomas but instead clustered in specific carcinomas suggesting that there is a CpG island hypermethylator phenotype in a subset of pancreatic adenocarcinomas (CIMP+).

IPMN (intraductal papillary mucinous neoplasm) is a non-invasive cystic neoplasm characterized by unique clinical, pathologic and molecular features. IPMN exhibit a wide spectrum of malignant potential and accurate preoperative assessment of the grade of malignancy is critical to determine the optimal management and furthermore conventional cytologic analysis and serum Ca 19.9 are not able to differentiate benign and malignant IPMN. The identification and characterization of overexpressed genes as well as unexpressed ones in IPMN might allow us to identify clinically relevant biomarkers. Pancreatic endocrine neoplasms (PENs) are a unique group of malignancies often characterized by a clinical neuroendocrine syndrome with overproduction and circulation of pancreas-specific hormones. DNA methylation of tumor suppressor genes promoter sites predicts tumor behaviour and a unique methylation pattern associated with PEN shows ability to predict clinical outcome after surgical resection.

Normal, neoplastic tissues and patients' serum were obtained from 24 pancreatic adenocarcinomas, 3 IPMN and 4 PEN resected at Surgical Oncology Unit, Siena from Jan 2001 to Sep 2006 and stored at -70°C . Data were stored with TNM OS DFS LS and median follow-up.

The methylation status of 5' CpG islands by bisulfite modification of DNA and methylation specific PCR (MS-PCR) will be performed according to the method of Herman et al., carried out for 16 h at 50° on 1 μg of genomic DNA at Surgical Oncology Unit Laboratory in order to define:

- A subgroup of CIMP+ in pancreatic adenocarcinomas and its correlation to demethylators drugs use in chemotherapy.
- Hypermethylation correlation to IPMN evolution.
- Methylation pattern associated with PEN outcomes predictions after surgical resection.

References

1. Bayliss S. et al. Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv. Cancer Res.* 1998
2. House M. et al. Aberrant hypermethylation of tumor suppressor genes in pancreatic endocrine neoplasms. *Annals of Surgery*, 2003
3. Ueki T. et al. Hypermethylation of multiple genes in pancreatic adenocarcinoma. *Cancer Research*. 2001
4. Kawai M, et al. Clinicopathological features of malignant intraductal papillary mucinous tumors of the pancreas: the differential diagnosis with benign entities. *Arch Surg*, 2004
5. Herman J. et al. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc. Natl. Acad. Sci.* 1996

Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
Federica Ottimo, BS
 Tutor A. Renieri



Rett syndrome: MECP2 genotype-phenotype correlation

Rett syndrome (RTT) is an X-linked progressive neurodevelopmental 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 behaviour in girls. In addition to the classic form, five distinct RTT variants have been described. Among the RTT variants the preserved speech variant (PSV) is the most common in our series, while others such as “forme fruste”, the congenital and 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 other variants (40-50%). We analyzed the MECP2 gene in 213 RTT patients (detailed clinical information are collected in the RTT database; <http://www.biobank.unisi.it>) for point mutations and for deletions, by DHPLC and Real Time qPCR, respectively. A MECP2 mutation was identified in 162 patients: in 113 out of 126 unrelated classic RTT cases (89,7%) and in 27 out of 51 RTT variants (53%) (table1). The aim of our current study is to establish a genotype/phenotype correlation in RTT. First of all we have classified mutation types on the basis of their effects on MeCP2 function: missense mutations (located in MBD or TRD), early truncating mutation (causing TRD and/or MBD loss), late truncating mutations (causing C-terminal domain loss) and gene deletions. As summarized in table 2, all mutation types have been identified in classic RTT patients, while in PSV patients only missense mutations and late truncating mutations occur. These results support the notion that early-truncating mutations by causing complete loss of MeCP2 function are preferentially associated with a more severe phenotype. On the other hand, missense and late-truncating mutations which do not abolish completely function may be associated with a less severe phenotype. The number of the MECP2 mutated patients with the other variants is too small to draw any conclusion. The next goal of this work is to speculate a possible genotype-phenotype correlation among the classic RTT group by collecting detailed clinical features and applying a clinical score of severity to each patient.

Table 1:

PHENOTYPE	NEGATIVE MECP2 MUTATED	Mut. Detect. Rate	TOT	
Classic RTT	13	113	89.7%	126
Variants	24	27	53%	51

Table 2:

PHENOTYPE	Missense mutation	Early truncating	late truncating	gene deletions	TOT
Classic	30	60	13	10	113
PSV	12	0	8	0	20
Forme fruste	2	1	1	0	4
Rett-like	3	1	1	0	5
hPSV	1	0	1	1	3
congenital	0	0	0	0	0



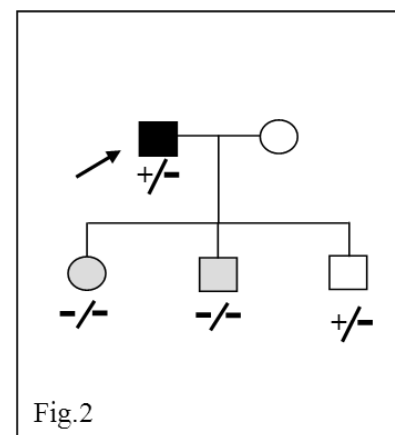
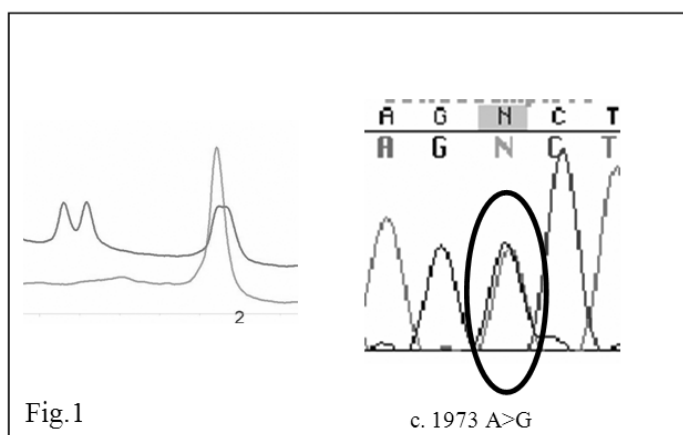
Oncology and Genetics Doctoral School
 Medical Genetics
 XXII cycle
Filomena Tiziana Papa, BS
 Tutor A. Renieri

WDR36 is not a glaucoma gene

The term primary glaucoma describes a heterogeneous group of optic neuropathies that lead to optic nerve atrophy and permanent loss of vision. It is the second most prevalent cause of bilateral blindness in the Western world. Primary Glaucoma includes various clinical entities, such as ocular hypertension (OH), glaucoma with elevated intraocular pressure (POAG) and normal tension glaucoma (NTG). Genetic factors play a major role in the aetiology of POAG. Up to now 9 loci (GLC1A > GLC1I) have been associated with POAG using linkage analysis. Some POAG families have mutations in the genes associated to GLC1A, GLC1E and GLC1G loci. Mutation in the Myocillin gene (MYOC) at locus GLC1A, have been reported in 2-4% of POAG patients and in up to 33% of JOAG patients. In 2002, the Optineurin gene (OPTN), associated to GLC1E locus was identified. In the 54 families analysed with adult-onset POAG and at least one member with NTG, OPTN mutations have been found in 16.7% of cases. In 2005 a new locus on chromosome 5q22 related to POAG and NTG was identified. It was also identified the associated gene, named WDR36. WDR36, a member of WD40 repeat protein family, has been proposed as the third gene of the primary open angle glaucoma, in addition to MYOC and OPTN genes.

To assess the involvement of WDR36 mutations in autosomal dominant POAG in Italian patients, we analyzed the gene in a cohort of 34 Italian families with autosomal dominant POAG by Denaturing High-Performance Liquid Chromatography (DHPLC). Among the 34 families enrolled, 25 were affected by POAG, 4 by JOAG and 1 by NTG. In addition, 4 families presented within the same pedigree both JOAG and POAG patients. Four already known intronic polymorphisms (IVS5+30C>T; IVS12+90 G>T; IVS13+89G>A; IVS16-30A>G) and a novel one (IVS21-75G>A), have been identified. One proband was found to carry the D658G mutation (Fig.1), reported as the more recurrent disease-causing allele. Surprisingly, the mutation does not segregate with the disease in the family excluding its pathogenic role (Fig.2). Although a role of WDR36 as a susceptibility gene in non mendelian open angle glaucoma can not be excluded, the results of this study question the involvement of WDR36 gene as a disease-causing gene in mendelian open angle glaucoma.

To assess the role of different genes in the pathogenesis of autosomal dominant glaucoma, we will analyze by DHPLC the MYOC gene in our cohort of POAG patients and in a group of 35 NTG patients.



Oncology and Genetics Doctoral School
Oncological Genetics
XXII cycle
Flavio Rizzolio, BS
Tutor A. Giordano



Defining the role of pRB2/p130-E2F4/5, STAT3 and ER β in Ovarian Surface Epithelium (OSE) and Ovarian Carcinoma (OC) by ChIP on chip analysis

The importance of understanding the molecular pathway involved in the normal cell life such as proliferation, quiescence, apoptosis and senescence is becoming of great importance in order to dissect cancer pathology as reported by several recent papers. Microchip analyses have discovered thousand of genes whose expression was altered in cancer cells compared to normal tissues. Unfortunately, from these analyses it is very difficult to find the few master genes that regulate many others target genes.

In order to increase our knowledge on some of the mechanisms leading to cancer I propose the use of epithelia cell lines from benign and ovarian cancer with the application of ChIP on chip technology, bioinformatic approach and shRNA/overexpression system to investigate the function of genes whose deregulation may lead to the development of cancer.

pRB2/p130-E2F4/5, STAT3 and Estrogen Receptors (ERs) are transcription factors which bind to a lot of promoters of genes involved in different cellular processes and in particular proliferation and migration. The interaction between these proteins and gene target promoters is regulated by modifications of histone tails. Chip on chip technology will allow us to identify which genes are controlled from these transcription factors and how the chromatin modifications are determinant for the binding of DNA. Because pRB2/p130-E2F4/5, STAT3 and ERs are largely known to be involved in different type of cancer, the target genes could potentially play a role in the progression of ovarian cancer. On the other hand, these analyses will provide us with the information of different pathways in which these genes are involved. Quantitative RT-PCR in different OC cell lines and tissue biopsies in combination with bioinformatic databases will be utilized to test the expression of target genes. The most interesting genes will be further examined using Short Hairpin RNA (shRNA) and overexpression systems to confirm their role in the pathology. Moreover, three animal model of ovarian cancer are now been developed and they would be helpful to elucidate the cancer signature in vivo. The proposed studies will lead to the identification of novel genes important for tumor cell proliferation and migration, two important steps for cancer progression. Since my long-term goal is the improvement and discovering of new drugs, my first aim is to understand if these target genes are involved in the drugs resistance phenomenon that now makes the treatment ineffective.



Medical Genetics Doctoral School
Oncological Genetics
Annalisa Roberti, BS
XX cycle
Tutor A. Giordano

Characterization of novel sporadic Burkitt lymphoma cell line (GAL 1): analysis of cell cycle regulation proteins and pRB2/p130 pathways

The World Health Organization Classification of Lymphoid Neoplasms identifies Burkitt lymphoma (BL) as a highly aggressive mature B-cell neoplasm consisting of endemic (eBL), sporadic (sBL), and immunodeficiency-associated (AIDS-related BL) variants. These subtypes share many morphologic and immunophenotypic features, but differences exist in their clinical and geographic presentations. All of these subtypes possess chromosomal rearrangements of the c-myc oncogene, the genetic hallmark of Burkitt lymphoma, that contributes to lymphomagenesis through alterations in cell cycle regulation, cellular differentiation, apoptosis, cellular adhesion, and metabolism. Human cell lines constitute a very useful tool to investigate the biology of lymphoid neoplasms. However, few sBL cell lines have been submitted to detailed cytogenetic characterization. Recently, a novel sBL cell line was established from a HIV-negative patient with an Epstein-Barr virus (EBV)-negative sporadic BL, presenting as an effusion (Thielen C et al.). This cell line was referred to as GAL1 cell. GAL1 cells display the phenotype and genotype of B-cell lineage (CD20+, CD79+a and presenting clonal rearrangement of Ig heavy chain), they carry the c-myc rearrangement by t(8;22)(q24;q11) translocation and are characterised by the expression of the germinal centre-associated antigens CD10, BCL6, CD38 and show low BCL2 expression. Subcutaneous injection of this cell line in mice induced tumour formation. It should also be considered that the c-myc translocation is not the only genetic lesion found in BL and that c-myc-transformed cells are usually characterized by the loss of expression of several genes. Cooperating alterations of cell cycle-associated genes may contribute to the pathogenesis of BL. Genetic alterations disrupting the nuclear localization of the retinoblastoma-related gene RB2/p130 have been documented in BL cell lines as in primary tumors. Recently, point mutations of RB2/p130 have been described, leading to amino acid substitutions in the COOH terminal domain of the protein, namely in the bipartite NLS. Cells bearing such mutations produce a full-length pRb2/p130 molecule, which remains consistently confined to the cytoplasm. The final result is the failure to reach a satisfactory intracellular localization, with consequent inability to negatively modulate the cell cycle, by physical interference with nuclear transcription factors.

The aim of my research was to evaluate the intracellular distribution of pRb2/p130 in the the GAL1 cell line, to assay if this tumor suppressor gene may have a role in the evolution and progression of sBL.

To study subcellular localization of pRb2/p130 an Immunofluorescence staining in the Gal1 cell line, using a monoclonal antibody against the NH2-terminal region of pRb2/ p130, was performed. As a control of a normal nuclear localization of pRb2/p130, a human B-lymphoblastoid cell line, immortalized with EBV, but not transformed (EBV-B), was used. As depicted in Fig. 1, GAL1 cell lines show a prevailing localization of the protein at the cytoplasmic level, whereas EBV-B cells exhibits an exclusive nuclear localization of pRb2/p130. This result was further supported by Western blotting analysis after cell fractionation. pRb2/p130 was detected both in the cytoplasm and nucleus confirming the subcellular localization demonstrated by the Immunofluorescence staining. To confirm the purity of the cell fractionation, the subcellular fractions were also analyzed by Western blotting with the cytoplasmic marker α -tubulin and the nuclear marker Sp1 proteins. As shown in Fig 2, no cross-contaminations between the nuclear and the cytoplasmic fraction were observed. In previous studies a cytoplasmic staining of the pRb2/p130 protein was ascribed to a guanosine or a cytosine insertion at exon 21, which caused the loss of the bipartite nuclear localization signal (NLS), resulting from a shift in the coding frame with a consequent stop codon upstream of the NLS. Therefore was performed a DNA-sequencing analysis of exon 21. An insertion of an A within the splice site of exon 21 was observed which may be responsible for the altered expression of pRb2/p130 in this cell line. It remains to be proven whether and how this impaired pRb2/p130 localization may be relevant for GAL1 neoplastic transformation and/or may give the cells a more aggressive malignant phenotype.

Oncology and Genetics Doctoral School
 Medical Genetics
 XXII cycle
Ariele Spanhol Rosseto, BS
 Tutor A. Renieri

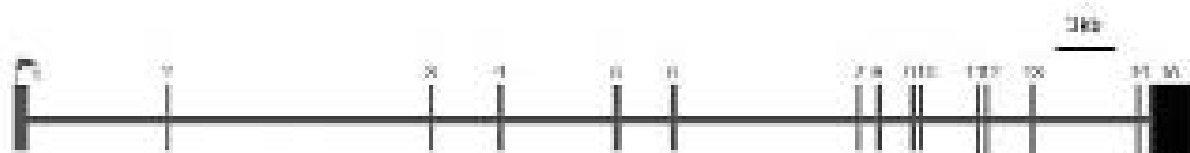


Polycystic Kidney Disease

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a disorder characterised by renal cysts development and enlargement resulting in bilateral polycystic kidneys and end-stage renal disease, typically in late middle age. It is clinically characterized by acute hypertension, and extrarenal manifestations including liver cysts, ruptured intracranial aneurysms, cardiac valvular disease, colonic diverticula, dilatation of the aortic root and dissection of the toracic aorta, mitral valve prolapse, and abdominal wall hernias. The disease is diagnosed by ultrasonography and tomography or molecular genetic testing. ADPKD is caused by genetic defects in at least two different genes, PKD1 and PKD2, located on chromosomes 16 and 4, respectively. These two genes are quite large and pseudogenes are present making direct mutation analysis difficult. For this reason genetic diagnosis is usually performed by linkage analysis only in familiar cases. But this method does not permit diagnosis of sporadic cases and has a risk of false positive and false negative results. The aim of my project will be to set up the conditions for direct mutation analysis of PKD1 and PKD2 genes. We will start the analysis with PKD2 gene on 26 familiar cases, for one of which linkage to PKD2 was demonstrated. Subsequently the analysis will be widened to PKD1 gene.



PKD1 Gene



PKD2 Gene



Medical Genetics Doctoral School
Medical Genetics
XX cycle
Katia Sampieri, BS
Tutor A. Renieri

Searching for modifier genes in retinoblastoma

Retinoblastoma (RB, OMIM#180200) is the most common primary intraocular malignancy in children, caused by a two-step inactivation of both alleles of the RB1 gene. In retinoblastoma, 80% to 90% of RB1 mutation carriers develops multifocal eye tumours, most often bilaterally. However, we observed a broad range of phenotypic variability among RB patients (age of tumour onset, involvement of one or two eyes, tumour dimension and level of therapy necessary for remission as outcomes). We hypothesized that the variability of RB phenotype is the result of the level of function of genes involved in cell cycle progression and apoptosis. We firstly decided to investigate the association between the presence of polymorphisms in candidate genes with the age at tumour diagnosis. In particular, by using Pyrosequencing AB, we genotyped functional polymorphisms in MDM2 and TP53 genes in a group of 69 RB patients, with a known or suspected RB1 germline mutation.

We first analyzed an SNP in exon 4 of TP53 (rs1042522:G>C) which has been demonstrated to decrease the apoptotic potential of the protein and to influence prognosis in squamous carcinoma. Using univariate analysis, we obtained that the presence of this SNP does not influence age of diagnosis in RB patients. We then analyzed an SNP located in the MDM2 gene promoter (rs2279744:T>G), which as been showed to be associated with accelerated tumour formation in both hereditary (Li-Fraumeni syndrome) and sporadic cancers (colorectal cancer). On the contrary, in our group of patients, Kaplan-Meier survival curves (Fig.1) showed that the genotype G/G is associated with a delayed tumour onset compared with T/G and T/T genotypes.

An increased number of patients together with the use of multivariate analysis, will allow to firmly establish if such association does exist. This results could lead to define a more accurate prognosis for RB patients.

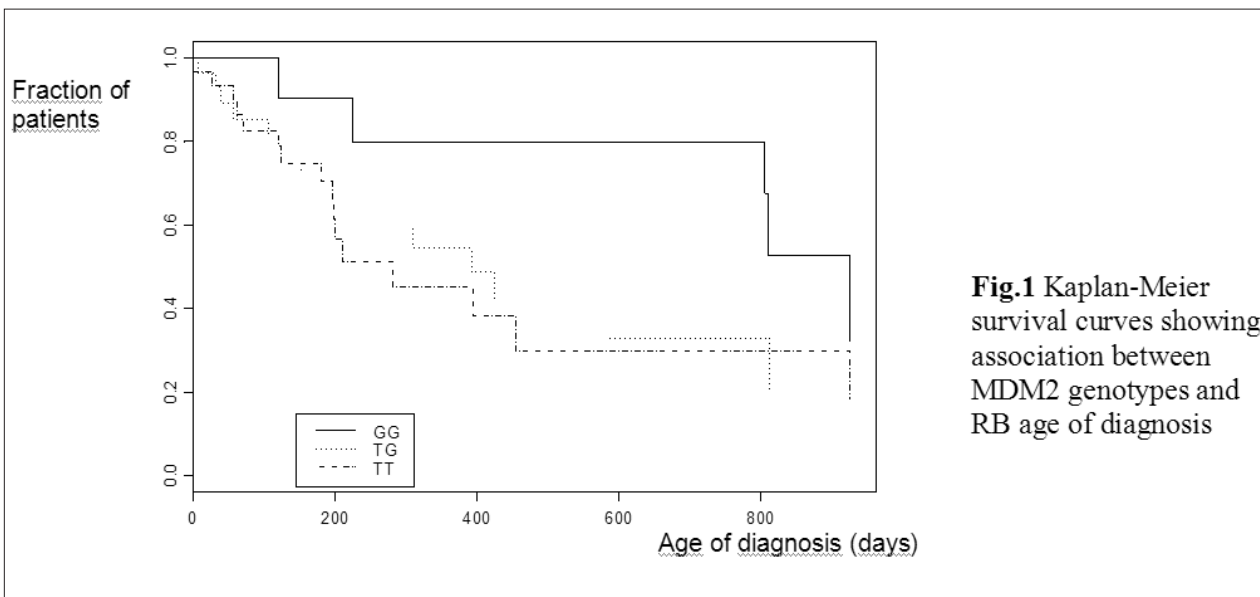


Fig.1 Kaplan-Meier survival curves showing association between MDM2 genotypes and RB age of diagnosis

Part of this work is published in: Sampieri K, et al. Mutational screening of the RB1 gene in Italian patients with retinoblastoma reveals 11 novel mutations. J Hum Genet. 2006;51(3):209-16.

Doctorate in Medical Genetics
 XIX cycle
Elisa Scala, BS
 Tutor A. Renieri



Italian Rett Database and biobank

Rett Syndrome (RTT, OMIM#312750) is a neurodevelopmental disorder that predominantly affects females, with an incidence of approximately 1/10.000-15.000 live female births. In addition to the classic form, a number of RTT variants have been described. MECP2 gene mutations are responsible for about 90% of classic cases and for a lower percentage of variant cases. Recently, our group and others identified mutations in the CDKL5 gene in patients with a diagnosis of early seizures variant and in other atypical RTT patients. While the high percentage of MECP2 positive classic RTT patients supports the hypothesis of a single gene causing this phenotype, the low frequency of mutated RTT variant cases implies genetic heterogeneity for RTT variants, as already demonstrated by CDKL5 mutated cases. The RTT biobank is active since 1998 to collect detailed clinical information and biological samples from a vast number of RTT patients throughout Italy. Collected samples and clinical information are inserted within an on-line database available at the address <http://www.biobank.unisi.it>. Given the clinical variability among classic RTT girls and the hypothesized genetic heterogeneity among variants, the availability of a considerable number of patients with accurate molecular and phenotypic classification is a crucial factor for researchers working on RTT. The information available in the bank will permit a detailed correlation between particular clinical features and specific mutations. Furthermore, the sharing of clinical and molecular data and of biological samples will help to identify new RTT causative genes and to better define the pathogenic mechanisms underlying this condition. Given that for rare disorders such as RTT, the Internet provides an important means of communication, this on-line database will surely encourage collaboration with clinicians and researchers from all around the world.

a

MECP2
 list of mutations
 graph of mutations
 Rare variants

CDKL5
 list of mutations
 graph of mutations
 Rare variants

Search by
 Mutated Gene:
 MECP2: CDKL5: Unknown:
 Survival
 Mutation Type
 Nucleotide Change
 AA Change
 Phenotype

b

AA Change

c

Code	Internal Code	Phenotype	Mutated Gene	Mutation Type	Nucleotide Change	AAChange	Additional Info
2	4, proband 5, mother 6, Father	classic Rett	MECP2	early truncating	808C>T	R270X	
12	39, proband 37, Mother 36, Father	classic Rett	MECP2	early truncating	808C>T	R270X	
18	51, proband	classic Rett	MECP2	early truncating	808C>T	R270X	

Part of this work is published in: Sampieri K, Meloni I, Scala E, et al. Italian Rett database and biobank. Hum Mutat. 2007 Apr;28(4):329-35.



Medical Genetics Doctoral School
Medical Genetics
XX cycle
Caterina Speciale, MD
Tutor A. Renieri

Searching the mental retardation gene in 13q14 deletion syndrome (Retinoblastoma and MR)

The 13q deletion syndrome is an uncommon but well-recognized abnormality of chromosome 13 that was described for the first time in 1983 by Motegi et al. Although the phenotype associated with this contiguous genes deletion syndrome has been variable, a pattern of malformations has emerged that allows easier clinical suspicion of this chromosomal imbalance. Usually, it is characterized by retinoblastoma associated with psycho-motor developmental delay, dysmorphic features and structural malformations of the brain and other organs and systems. Peculiar cranio-facial anomalies are: frontal bossing, depressed and broad nasal bridge, bulbous tip of the nose, deeply grooved and long philtrum, thin upper lip, thick lower lip, broad cheeks and large ears and lobules. The wide spectrum and variability of manifestations depend on how much of the long arm of chromosome 13 is deleted.

In a first phase of the study we have clinically characterized 3 patients with retinoblastoma associated with psycho-motor developmental delay and dysmorphic features. Case 1, a 1y 6m-old female, and case 2, a 2y7m-old male, have a similar facial appearance with high and broad forehead, deeply grooved philtrum, and thick anteverted lobes and helix. In addition, Case 1 presents dolicocephaly, sacral pit and toe crowding. Case 2 has severe intrauterine growth retardation and short fifth toe. Both case 1 and case 2 have partial agenesis of corpus callosum. Case 3 is a 7 year 6 months old female with mild to moderate growth retardation, severe microcephaly, thick lower lip and micrognathia.

Given the presence of a peculiar phenotype, we decided to apply array-based Comparative Genomic Hybridization (Array-CGH) to investigate chromosomal imbalances in these patients.

Array-CGH experiments allow us to identify and characterize a 13q deletion in both Case 1 and 2 of about 19 and 36 Mb, respectively [Case 1: 46,XX,del(13)(q13.3q21.2); Case 2: 46,XY, del(13)(q14.11q31.1)]. Case 3 has a 7q deletion inherited from the normal mother and probably not related to the disease. Array-CGH results were confirmed by real-time quantitative PCR.

Deletions identified in Case 1 and 2 partially overlap and allow us to define a wide critical region of almost 16 Mb which includes 39 known genes. In order to restrict the critical region and to identify candidate genes for mental retardation and dysmorphisms, we plan to analyze additional patients with isolated retinoblastoma and deletion of the RB1 gene. At present, we have analyzed by array-CGH a patient with isolated retinoblastoma and with Rb1 deletion previously identified by real time quantitative PCR. This patient shows a smaller 13q deletion (about 1.8 Mb) included in the critical region identified. In this region are located 14 known genes that we can exclude as responsible for mental retardation and dysmorphisms in the 13q14 deletion syndrome. Molecular characterization of other patients affected by isolated familial retinoblastoma with bigger deletion than this one may allow us to restrict further on the critical region.

Part of this work is published in: Caselli R, Speciale C, et al.

Retinoblastoma and mental retardation microdeletion syndrome: clinical characterization and molecular dissection using array CGH. J Hum Genet. 2007;52(6):535-42.

Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
Tiziana Squillaro, BS
 Tutor A. Renieri



LRRK2 mutation analysis in patients affected by Parkinson's disease

Parkinson's disease (PD; MIM 168600) is the second most common neurodegenerative disorder after Alzheimer disease affecting more than 1% of individuals aged 55 years. Neuropathological features include loss of dopaminergic neurones in the substantia nigra-pars compacta and other brain regions, with formation of ubiquitin containing inclusion in surviving neurones.

An autosomal dominant form of parkinsonism (PARK8) was first mapped to chromosome 12 in a Japanese family. This linkage was successively confirmed in Caucasian families and mutations in the gene Leucine-Rich Repeat Kinase (LRRK2) have been identified in PARK8 linked families.

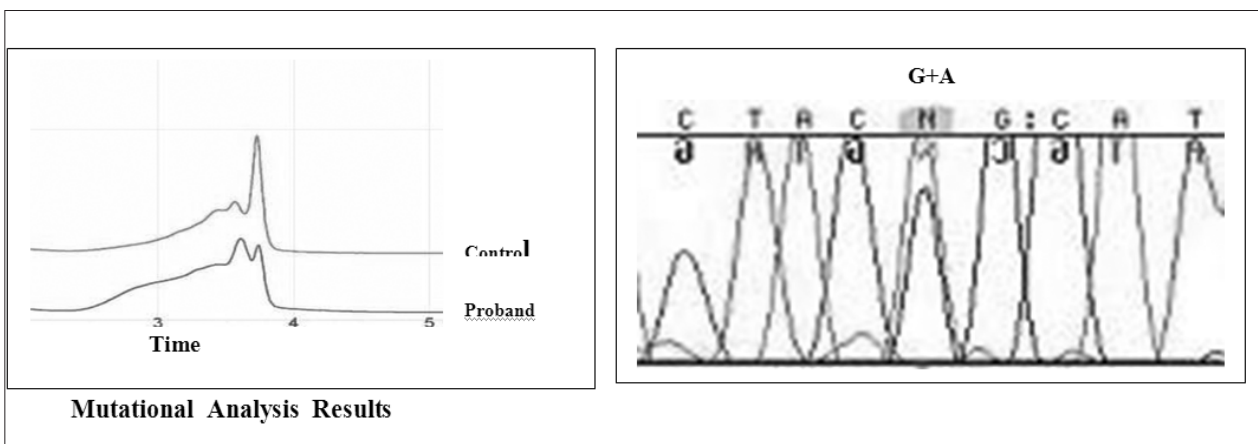
Until now, several different missense mutations have been identified in the LRRK2 gene. The G2019S substitution in exon 41 is extremely common in populations of European origin, accounting for approximately 5% to 6% of familial and 0.5% to 2% of apparently sporadic cases, depending on the geographic location.

The aim of my study was to determine the frequency of the LRRK2 G2019S mutation in Italian patients with idiopathic PD who have been referred to the Medical Genetics of the University of Siena.

I performed the analysis of the LRRK2 G2019S mutation in 98 unrelated PD patients, including 12 probands belonging to families compatible with autosomal dominant inheritance (12%) and 86 sporadic cases (88%). I detected the mutation in one sporadic case (1.2%). The patient is a 53 years old female who developed PD symptoms at the age of 47 years.

These results confirm previous studies that demonstrated a frequency of about 1% for the G2019S mutation in the sporadic cases of idiopathic PD in the Italian population.

Since genetic predisposition is important in the pathogenesis of PD and the G2019S LRRK2 mutation contributes to a significant percentage of cases, screening for this substitution may become a key component of genetic testing for PD in the near future. As already underlined in previous studies, such genetic test should be extended also to sporadic cases. The identification of a frequent genetic determinant of PD has also important implications for genetic counselling.



Part of this work was published in: Squillaro T, et al. Frequency of the LRRK2 G2019S mutation in Italian patients affected by Parkinson disease. J Hum Genet. Submitted revised version



Oncology and Genetics Doctoral School
 Medical Genetics
 XXI cycle
Marina Vignoli, BS
 Tutor M. Genuardi

Role of CDKN2A deletions in predisposition to familial melanoma

Familial cutaneous melanoma accounts for about 10% of all melanoma cases and is inherited as an autosomal dominant trait, albeit with incomplete penetrance and variable expressivity. Genetic linkage analyses in large familial melanoma kindreds have identified a melanoma susceptibility locus on human chromosome band 9p21. This region harbors the INK4 gene cluster consisting of the CDKN2A and CDKN2B genes. CDKN2A encodes two distinct proteins translated, in alternate reading frame, from alternatively spliced transcripts, the alpha transcript, comprising exons 1, 2, and 3, encodes p16 INK4a, while the smaller beta transcript, comprising exons 1, 2, and 3, specifies the alternative product p14ARF. The p16 protein inhibits the activity of the cyclin D1-cyclin-dependent kinase 4 (CDK4) or 6 (CDK6) complexes that phosphorylate the retinoblastoma protein, thus p16 acts as a tumor suppressor and negatively regulates cell growth by arresting cells at G1. p14ARF acts via the p53 pathway to induce cell cycle arrest or apoptosis.

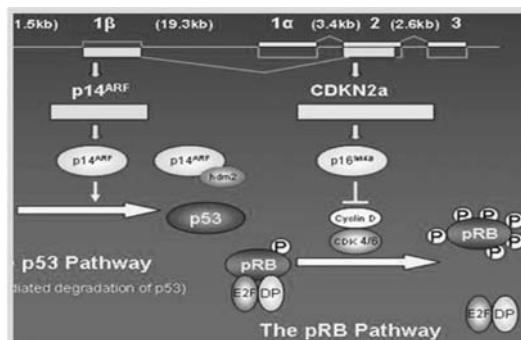
Germline mutations in INK4 locus are associated with melanoma susceptibility in 20–40% of multiple case melanoma families. Many of these mutations specifically impair p16INK4a, whereas mutations uniquely targeting p14ARF are rare. Although the CDKN2A gene has been shown to be the major genetic determinant governing high-penetrance susceptibility to melanoma, there remains a significant proportion of melanoma pedigrees in which germline mutations of CDKN2A have not been identified.

To determine the contribution of these candidate genes to familial melanoma genetic predisposition, we have studied the presence of germline deletions encompassing the CDKN2A locus in melanoma pedigrees.

To investigate the possibility that CDKN2A is affected by germline deletions in patients who do not show point mutation, we performed quantitative analysis of this locus using two independent methods, based on MLPA (multiplex ligation-dependent probe amplification) and real-time quantitative PCR using sybr green. The former investigates multiple sequences clustered on chromosome 9p21, including the CDKN2A promoter regions and the CDKN2B locus, while for the latter we initially set up analytical conditions limited to CDKN2A coding regions.

At the moment we have analysed 6 unrelated cases. With both methods, no deletions targeting CDKN2A coding regions were identified; however, MLPA analysis revealed a 50% reduction in the area of the peak corresponding to the promoter region located upstream of CDKN2A exon 1.

Further analysis will be required to confirm this result and understand its significance. In addition, further available samples from melanoma families will be investigated for quantitative deletions.

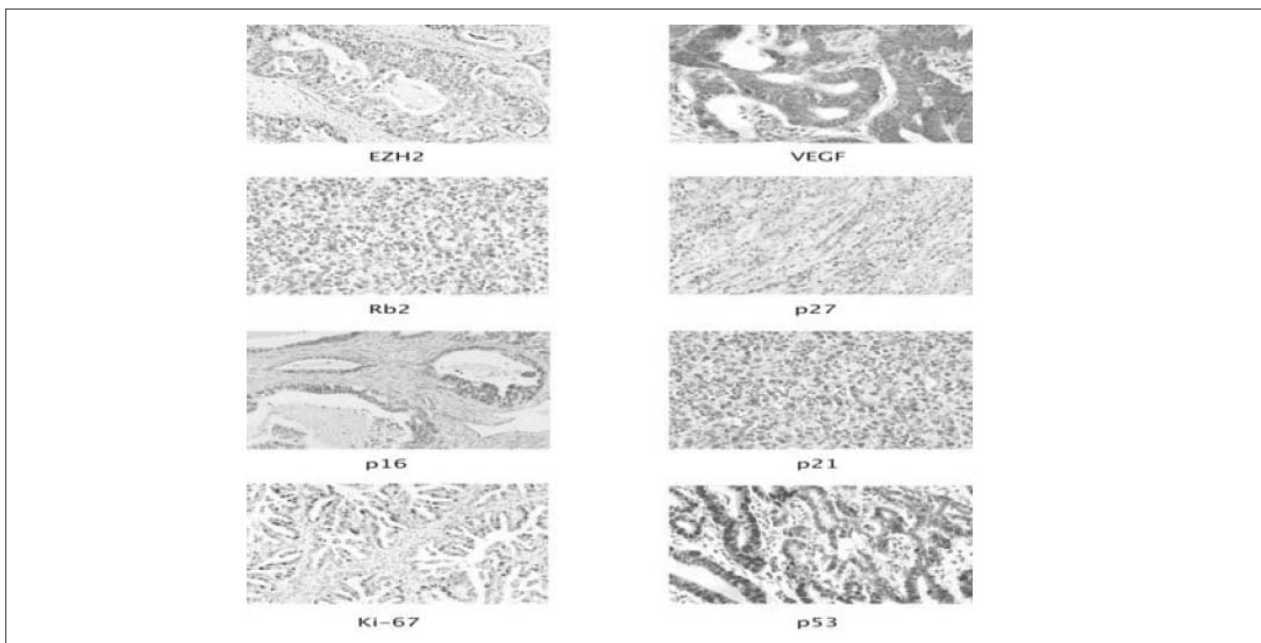


Doctorate in Medical Genetics
XVIII cycle
Paraskevi Vogiatzi, MD
Tutor A. Giordano



Immunohistochemical analysis of pRB2/p130, VEGF, EZH2, p53, p16INK4A, p27KIP1, p21WAF1, Ki-67 expression patterns in gastric cancer

Although there is considerable progress in gastric cancer research, it remains a complex lethal disease defined by peculiar histological and molecular features. The purpose of the present study was to investigate pRb2/p130, VEGF, EZH2, p53, p16INK4A, p27KIP1, p21WAF1, Ki-67 expressions, and analyze their possible correlations with clinicopathological factors. The expression patterns were examined by immunohistochemistry in 47 patients, 27 evaluated of intestinal-type, and 20 of diffuse-type, with a mean follow up of 56 months and by Western blot in AGS, N-87, KATO-III, and YCC-2, -3, -16 gastric cell lines. Overall, stomach cancer showed EZH2 correlated with high levels of p53, Ki-67, and cytoplasmic pRb2/p130 ($P < 0.05$, and $P < 0.01$, respectively). Increased expression of EZH2 was found in the intestinal-type and correlated with the risk of distant metastasis ($P < 0.05$ and $P < 0.01$, respectively), demonstrating that this protein may have a prognostic value in this type of cancer. Interestingly, a strong inverse correlation was observed between p27KIP1 expression levels and the risk of advanced disease and metastasis ($P < 0.05$), and a positive correlation between the expression levels of p21WAF1 and low-grade (G1) gastric tumors ($P < 0.05$), confirming the traditionally accepted role for these tumor-suppressor genes in gastric cancer. Finally, a direct correlation was found between the expression levels of nuclear pRb2/p130 and low-grade (G1) gastric tumors that was statistically significant ($P < 0.05$). Altogether, these data may help shed some additional light on the pathogenetic mechanisms related to the two main gastric cancer histotypes and their invasive potentials.



Part of this work is published in:
Mattioli E, Vogiatzi P, et al. Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer. *J Cell Physiol* 2006 Sep 22; [Epub ahead of print]

Thesis discussion

Doctorate in Medical Genetics

November 20, 2007 room 6 Centro Didattico S. Maria alle Scotte, 14.30

14.30 Entering of the PhD dissertation board composed by

- Prof. Salvatore Oliviero, (President)
Professor of Molecular Biology, University of Siena, Italy
- Prof. Sergio Ferrari, (Member)
Professor of Biology, University of Modena e Reggio Emilia, Italy
- Prof. Gianpaolo Papaccio (Member)
Professor of Istology, University of Napoli, Italy
- Prof. Antonio Giordano, (Secretary)
Professor of Pathology, University of Siena, Italy

14.45 Thesis discussion in English language

- “Expression analysis of key trigger genes in gastric cancer: from etiology and subtyping to early prognostic assessment”, Paraskevi Vogiatzi cycle XVIII

15.45 Thesis discussion in English language

- “Functional analysis of PIM1 kinase and its role in vasculogenesis”, Alessandra De Robertis cycle XVIII

16.45 Awarding of the PhD degree in Medical Genetics

17.00 End of session

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.

Thesis discussion

Doctorate in Medical Genetics

June 11, 2007 room 6 Centro Didattico S. Maria alle Scotte, 16.00

16.00 Entering of the PhD dissertation board composed by

- Prof. Lidia Larizza, (President)
Professor of Medical Genetics, Milan, Italy;
- Prof. Sabrina Giglio, (Member)
Professor of Medical Genetics, Florence, Italy;
- Prof. Franco Laccone, (Member)
Professor of Medical Genetics, Vienna, Austria;
- Prof. Alessandra Renieri, (Secretary)
Professor of Medical Genetics, University of Siena, Italy.

16.15 Thesis discussion in English language

- "Multiple congenital anomalies and mental retardation: analysis by oligo array-CGH",
Rossella Caselli cycle XIX

17.15 Thesis discussion in English language

- "Molecular bases of clinical variability in Rett syndrome", Elisa Scala cycle XIX

18.15 Evaluation of both candidates for qualification of Doctor Europaeus

The PhD dissertation board took into account the report of the external PhD theses reviewers, who were:

- Dr. Nigel Carter, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom;
- Prof. Dian Donnai, Medical Genetics, University of Manchester, United Kingdom;
- Dr. Gerard Hilaire, Centre National de la Recherche Scientifique, Marseille, France;
- Dr. Merchè Pineta Marfà, Neuropediatria, Hospital Sant Joan de Deu, Barcelona, Spain.

18.45 Awarding of the PhD degree in Medical Genetics and of the qualification of Doctor Europaeus

19.00 End of the session

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".

Doctor Europaeus

Awarding of qualification in Medical Genetics

June 11, 2007 room 6 Centro Didattico S. Maria alle Scotte, 16.00



From left to right
Elisa Scala
Prof. Franco Laccone
Prof. Lidia Larizza
Prof. Sabrina Giglio



From left to right
Prof. Alessandra Renieri
Prof. Franco Laccone
Prof. Lidia Larizza
Prof. Sabrina Giglio
Rossella Caselli

