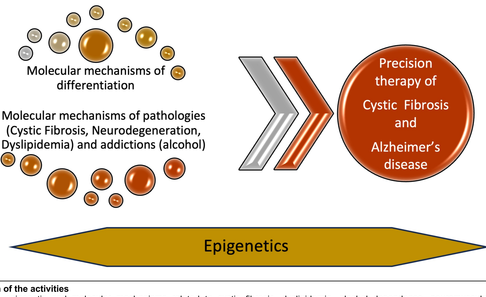
Clinical Biochemistry and Clinical Molecular Biology



General description of the activities
Our lab studies the epigenetic and molecular mechanisms related to cystic fibrosis, dyslipidemia, alcohol dependence, neuromuscular differentiation and neurodegeneration. For some of these pathologies, epigenetic, theratyping and gene editing therapeutic approaches are being evaluated. Diagnostic, therapeutic and healthcare support in the areas of Clinical Pathology, Clinical Biochemistry, Hematology and Coagulation as the central laboratory of the AOU Policlinico Umberto I is provided.

Role	Name	Position	E-mail	Publications	Keywords
Lab head	Prof. Marco Lucarelli	Associate Professor	marco.lucarelli@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=7007012451	Cystic Fibrosis; epigenetics; theratyping; precision medicine; cell differentiation; alcohol dependence.
Lab members	Prof. Andrea Fuso	Associate Professor	andrea.fuso@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=6506472679	DNA methylation; Non-CpG methylation;one-carbon metabolism; microRNAs; neurodegeneration; microgravity.
	Prof. Giampiero Ferraguti	Researcher	giampiero.ferraguti@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=6506051367	Epigenetics; genetics of alcohol dependence;cystic fibrosis; gene expression.
	Dr. Giovanna Blaconà	Post-doc	giovanna.blacona@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=57211229589	Cystic Fibrosis; theratyping; cell differentiation; epigenetics.
	Dr. Silvia Francati	Specialization student	silvia.francati@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=57453618800	Epigenetics; genetics of alcohol dependence; gene expression.
	Dr. Mariarita Virgulti	PhD student	mariarita.virgulti@uniroma1.it	https://www.scopus.com/results/authorNamesList.uri?name=name&st1=virgulti&st2=mariarita&origin=searchauthorlookup#top	Cystic Fibrosis; theratyping; epigenetics.
	Dr. Sara Allushi	Trainee	sara.allushi@uniroma1.it	https://www.scopus.com/results/authorNamesList.uri?name=name&st1=allushi&st2=sara&origin=searchauthorlookup	Cystic Fibrosis; theratyping; epigenetics.
	Dr. Giancarlo Testino	Laboratory technician	giancarlo.testino@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=57191036179	Gene expression; epigenetics; DNA sequencing.
	Dr. Luiza Borges	PhD Student	luiza.borges@uniroma1.it	https://www.scopus.com/authid/detail.uri?authorId=57205731810	DNA methylation; one-carbon metabolism; microRNAs; neurodegeneration.
	Dr. Daniele Antinori	PhD student	daniele.antinori@uniroma1.it	https://www.scopus.com/results/authorNamesList.uri?name=name&st1=antinori&st2=daniele&origin=searchauthorlookup	DNA methylation; one-carbon metabolism; microRNAs; neurodegeneration.

Previous and current research

Epigenetics
The lab is actively involved in the study of the metabolism sustaining DNA methylation reactions, the "one-carbon" metabolism, by using modulators of this metabolic cycle, leading to hyper- or hypo-methylating status. Under these experimental settings, we specifically study the modulation of the non-CpG methylation and its functional role in regulating gene expression. The studies are aimed at unravelling the effects of the modulation on the different metabolites of the one-carbon metabolism and the effects that these alterations have on the cellular physiology. More recently we are also focusing on the cross-talk between DNA methylation and microRNAs expression.

Molecular mechanisms of differentiation
One of the epigenetic aspects studied in experimental murine neuromuscular systems is DNA methylation and its relationship with the transcriptional modulation of differentiation regulatory genes. The research concerns both the structural aspect, with studies on CpG and non-CpG methylation patterns, and the dynamic aspect of active and passive demethylation. Muscle satellite cells (C2C12), embryonic neural stem cells, as well as embryonic muscle and brain tissues are used.

Molecular mechanisms of some pathologies (Cystic Fibrosis, dyslipidemia) and addictions (alcohol)
A genetic disease studied is Cystic Fibrosis (CF, CFTR gene), with highly variable clinical manifestations and a complex relationship between genotype and phenotype. This has consequences for diagnosis, prognosis and therapy. This aspect is studied through extensive mutational analysis of CF patients with different clinical forms. The intra-CFTR and extra-CFTR genetic variability is related to the functional consequences of the variants found and the clinical aspect. Another group of genetic diseases studied are dyslipidemias. The genetic characterization and diagnosis of these pathologies are complex due to the numerous genes involved. With the aim of improving understanding of the molecular mechanisms underlying this group of pathologies, the mutational pattern of the LPL, ApoA1, LCAT, ABCA1 and LDL-R genes in patients is studied and the variants found are functionally characterized. The addiction studied is alcohol, with particular attention to its genetic basis. The gene investigated is the serotonin transporter (5-HTT), a molecular target of many antidepressants, which plays a fundamental role in the regulation of serotonergic neurotransmission. The research concerns the transcriptional regulation of 5-HTT in relation to epigenetic modifications and SNPs present in this gene.

Molecular mechanisms of neurodegeneration
The role of epigenetic mechanisms in neurodegenerative diseases is studied on human neuronal cell lines, on a mouse model of Alzheimer's disease (TgCRND8) and on human brain tissue from biobanks. The complex aetiology of Alzheimer's disease and other neurodegenerative pathologies highlights the possible involvement of epigenetic mechanisms in their development and progression, making the epigenetic approach useful both for understanding the molecular basis of the pathologies and for identifying possible therapeutic treatments or adjuvants. In the case of Alzheimer's disease, we have in fact been able to demonstrate how the alteration of the balance of the biochemical methylation pathway, dependent on the supply of B vitamins and homocysteine levels, could induce the onset of the Alzheimer-like phenotype in TgCRND8 mice, and how the administration of S-adenosylmethionine (the endogenous methylene agent) could achieve this phenotype. Over the last few years, we have identified some genes involved in the pathology of Alzheimer's and other neurodegenerative diseases (including the Presenilin 1 gene, PSEN1, and some proinflammatory interleukins, including IL-1B) whose expression is regulated by the methylation of their promoter. In preclinical experimental models, the methylation changes of these genes are used, together with other parameters characteristic of Alzheimer's pathology and neuroinflammation, as "markers" to evaluate the effectiveness of some natural molecules. S-adenosylmethionine, S-acetylglutathione and vitamin Kare currently being studied as possible molecules useful in the treatment of the pathology.

Theratyping, epigenetic and gene editing therapeutic approaches
One therapeutic approach pursued is the theratyping of CF, by testing patient-specific cell culture with specific rare mutated genotypes of CFTR, with already clinically used drugs. Another therapeutic approach is a repressive epigenetic intervention on genes coding for the epithelial sodium channel (ENaC), functionally related to CFTR and expressed at levels higher than physiological ones in CF due to lack of repression by CFTR. Another epigenetic therapeutic approach is the amplificatory therapy of CFTR expression by using DNA hypomethylating drugs. Finally, also SFHR gene targeting, capable of stably correcting a mutated genomic sequence by homologous replacement of a small fragment of wild-type DNA is tested for CF.

Selected Publications

- Orticello M, Cavallaro RA, Antinori D, Raia T, Lucarelli M, Fuso A. Amyloidogenic and Neuroinflammatory Molecular Pathways Are Contrasted Using Menaquinone 4 (MK4) and Reduced Menaquinone 7 (MK7R) in Association with Increased DNA Methylation in SK-N-BE Neuroblastoma Cell Line. Cells. 2023 Dec 27;13(1):58. doi: 10.3390/cells13010058. PMID: 38201262; PMCID: PMC10778373.
- Fiore M., Minni A., Cavalcanti L., Raponi G., Puggioni G., Mattia A., Garigliio S., Colizza A., Meliante P.G., Zoccali F., Tarani L., Barbato C., Lucarelli M., Ceci F.M., Francati S., Ferraguti G., Ceccanti M., Petrella C. The impact of alcohol consumption and oral microbiota on upper aerodigestive tract carcinomas: a pilot study. Antioxidants (Basel) (2023) 12(6):1233.
- Bruno SM, Blaconà G, Lo Cicero S, Castelli G, Virgulti M, Testino G, Pierandrei S, Fuso A, Cimino G, Ferraguti G, Eramo A, Lucarelli M. Quantitative Evaluation of *CFTR* Gene Expression: A Comparison between Relative Quantification by Real-Time PCR and Absolute Quantification by Droplet Digital PCR. Genes (Basel). 2023 Sep 9;14(9):1781. doi: 10.3390/genes14091781. PMID: 37761921; PMCID: PMC10531455.
- Raia T, Armeli F, Cavallaro RA, Ferraguti G, Businaro R, Lucarelli M, Fuso A. Perinatal S-Adenosylmethionine Supplementation Represses *PSEN1* Expression by the Cellular Epigenetic Memory of CpG and Non-CpG Methylation in Adult TgCRD8 Mice. Int J Mol Sci. 2023 Jul 19;24(14):11675. doi: 10.3390/ijms241411675. PMID: 37511434; PMCID: PMC10380323.
- Blaconà G., Raso R., Castellani S., Pierandrei S., Del Porto P., Ferraguti G., Ascenzioni F., Conese M., Lucarelli M. Downregulation of Epithelial Sodium Channel (ENaC) activity in cystic fibrosis cells by epigenetic targeting. Cellular and Molecular Life Sciences (2022) 79(5):257-274.
- Sette G., Lo Cicero S., Blaconà G., Pierandrei S., Bruno S.M., Salvati V., Castelli G., Falchi M., Fabrizzi B., Cimino G., De Maria R., Biffoni M., Eramo A., Lucarelli M. Theratyping cystic fibrosis in vitro in ALI-culture and organoid models generated from patient-derived nasal epithelial conditionally reprogrammed stem cells. European Respiratory Journal (2021) 58(6):2100908.
- Monti N, Cavallaro RA, Stoccoro A, Nicolia V, Scarpa S, Kovacs GG, Fiorenza MT, Lucarelli M, Aronica E, Ferrer I, Coppedè F, Troen AM, Fuso A. CpG and non-CpG Presenilin1 methylation pattern in course of neurodevelopment and neurodegeneration is associated with gene expression in human and murine brain. Epigenetics. 2020 Aug;15(8):781-799. doi: 10.1080/15592294.2020.1722917. Epub 2020 Feb 5. PMID: 32019393; PMCID: PMC7518704.
- Fuso A, Ferraguti G, Scarpa S, Ferrer I, Lucarelli M. Disclosing bias in bisulfite assay: MethPrimers underestimate high DNA methylation. PLoS One. 2015 Feb 18;10(2):e0118318. doi: 10.1371/journal.pone.0118318. PMID: 25692551; PMCID: PMC4333220.
- Pascale E., Ferraguti G., Codazzo C., Passarelli F., Mancinelli R., Bonvicini C., Bruno S.M., Lucarelli M., Ceccanti M. Alcohol dependence and serotonin transporter functional polymorphisms 5-HTTLPR and rs25531 in an Italian population. Alcohol and Alcoholism (2015) 50(3) 259-265.
- Lucarelli M., Bruno S.M., Pierandrei S., Ferraguti G., Stamato A., Narzi F., Amato A., Cimino G., Bertasi S., Quattrucci S., Strom R. A genotypic-oriented view of CFTR genetics highlights specific mutational patterns underlying clinical macro-categories of cystic fibrosis. Molecular Medicine (2015) 21:257-275.
- Fuso A., Ferraguti G., Grandoni F., Ruggeri R., Scarpa S., Strom R., Lucarelli M. Early demethylation of non-CpG, CpC-rich, elements in the myogenin 5'-flanking region: a priming effect on the spreading of active demethylation? Cell Cycle (2010) 9(19):3965-3976.
- Lucarelli M., Fuso A., Strom R., Scarpa S. The dynamics of myogenin site-specific demethylation is strongly correlated with its expression and with muscle differentiation. The Journal of Biological Chemistry (2001) 276(10):7500-7506.

Grants

- Gnosis by Lesaffre, grant for studies on S-adenosylmethionine and S-acetylglutathione in neurodegeneration and neuroinflammation
- MUR, PRIN 2022 "Theratyping of Cystic Fibrosis" (#2022FRSS2H)
- Istituto Pasteur Fondazione Cenci Bolognetti, The personalized therapy of Cystic Fibrosis by theratyping and gene targeting
- Fondazione per la Ricerca sulla Fibrosi Cistica (FFC), Theratyping of Cystic Fibrosis

Links

The Epigenetic Society - https://epigeneticssocietyint.com/home