MEDICAL BIOTECHNOLOGY AND NANOBIOTECHNOLOGY (LM49)

(Lecce - Università degli Studi)

Teaching CELLULAR BIOTECHNOLOGIES

GenCod A004552

Owner professor Cecilia BUCCI

Reference professors for teaching Cecilia BUCCI, ROBERTA ROMANO

Teaching in italian CELLULAR BIOTECHNOLOGIES

Teaching CELLULAR BIOTECHNOLOGIES

SSD code BIO/13

Reference course MEDICAL BIOTECHNOLOGY AND

Course type Laurea Magistrale

Credits 9.0

Teaching hours Ore-Attivita-frontale: 74.0

For enrolled in 2022/2023

Taught in 2022/2023

Course year 1

Language INGLESE

Curriculum PERCORSO GENERICO/COMUNE

Location Lecce

Semester Primo-Semestre

Exam type Orale

Assessment Voto-Finale

Course timetable https://easyroom.unisalento.it/Orario

BRIEF COURSE DESCRIPTION

Synthetic program:

Genetic manipulation of eukaryotic cells. Methods for expressing or silencing wt and mutant genes in cultured animal cells. Cellular and molecular biotechnologies to identify protein-protein interactions: the two-hybrid system and its variants, the phage display technology. Biology of stem cells: embryonic, foetal and tissue. Applications of stem cells. Induced pluripotent stem cells. Regenerative medicine. Gene therapy: vectors, methodologies and scopes. Cloning and therapeutical cloning.

Program of pratical laboratory classes:

Transfection of mammalian cells. Use of GFP to monitor transfection efficiency. Transfection of GFP-tagged proteins to establish their intracellular localization in mammalian cells. Immunofluorescence analysis. Differentiation of cultured mammalian cells.

REQUIREMENTS

No formal prerequisites or propedeuticity are required. However, a solid knowledge of cell biology, molecular biology and genetics is strongly recommended in order to be able to follow the course fully understanding the different topics

COURSE AIMS	The course aims to provide knowledge and skills to work professionally with roles of responsibility in the areas of medical biotechnology which make use of eukaryotic cells (wt or genetically modified). **Learning outcomes** Knowledge to be attained: ▪ Up to date technologies for genetic manipulation of cultured eucaryotic cells for gene function and therapeutical applications. ▪ Cellular and molecular biotechnologies to study and unravel molecular interactions ▪ Biology of embryonic and adult stem cell biology and of their therapeutical applications. ▪ Gene therapy approaches Abilities to be attained: ▪ Gene expression in mammalian cells ▪ Gene silencing in mammalian cells ▪ Use of reporter genes to monitor transfection efficiency, protein intracellular localization and protein activity.
TEACHING METHODOLOGY	Formal lectures (8 cfu= 64 hours) making use of slides and hypertext links to specific Web sites and pratical laboratory classes (1 cfu = 10 hours). Outside these activities, the students are expected to read assigned papers from scientific literature.
ASSESSMENT TYPE	The evaluation of the oral examination will be based on the level of the theoretical knowledge and practical abilities acquired through the description of topics and methodologies (70%), on the critical and problems solving abilities and (20%) and on communication skills (10%).

FULL SYLLABUS

Methods for expressing or silencing wt and mutant genes in cultured animal cells. Genetic manipulation of eukaryotic cells: principles and scopes. Stable and transient transfection. Plasmid vector for mammalian expression: characteristics. Viral vectors for mammalian expression: vectors based on vaccinia virus, BacMam systems, retrovirus, lentiviral vectors. Expression in COS cells. Constitutive and regulated expression. The Tet-ON and Tet-OFF systems. Reporter genes and selection markers. Silencing genes using oligonucleotides, PNAs, antisense RNA. RNA interference: mechanism and applications. siRNA and miRNA: physiological roles and applications. Other kinds of small RNAs to regulate gene expression. shRNA for stable silencing. Methods to introduce molecules in cells: Microinjection, calcium phosphate transfection, DEAE dextran transfection, lipofection, magnet-assisted transfection, dendrimers, nanorods, cell penetrating peptides (CPPs), receptor mediated transfection, etc.

Cellular and molecular biotechnologies to identify protein-protein interactions. The two-hybrid system: principles and applications. Vectors for the two-hybrid system. False positives and false negatives in the two-hybrid system. The two-hybrid system variants: the single hybrid, the three-hybrid, the RNA-based hybrid, the split-hybrid, the Ras-SOS system, etc. Two-hybrid system in bacterial and mammalian cells. The phage/virion-display: principles and applications. Vectors for phage/virion-display: M13 and baculovirus.

Biology of stem cells. Definition of stem cells. Embryonic, foetal and adult stem cells. Potency of stem cells. Plasticity of stem cells. Adult stem cells: hematopoietic stem cells, neural stem cells, epithelial stem cells and mesenchymal stem cells. Homing and engraftment of stem cells. Applications of stem cells. Induced pluripotent stem cells. Regenerative medicine and tissue engineering based on stem cells. Bone marrow transplant, corneal epithelial stem cell transplant, skin production and transplant, bone production and transplant, etc. The theory and the facts on cancer stem cells: implications for therapy.

Gene therapy. Principles of gene therapy. Principles of gene therapy for recessive monogenic diseases. Principles of gene therapy for acquired diseases. Gene therapy strategies. Modulating the immune system using gene therapy. Examples of gene therapy: successes and problems with ADA-SCID, X-linked SCID, chronic granulomatosis and epidermolysis bullosa gene therapy. Gene therapy for tumors and infectious diseases. Oncolytic vectors. *In vivo* and *ex vivo* gene therapy: advantages and disadvantages.

Animal cloning. The definition of cloning. Cloning methodology for mammals. Therapeutical cloning: technical and ethical problems in humans.

REFERENCE TEXT BOOKS

-BIOLOGIA CELLULARE E GENETICA: Parte Prima - Biologia Cellulare a cura di Fantoni, Bozzaro, Del Sal, Ferrari - Casa Editrice PICCIN.

-MOLECULAR BIOLOGY OF THE CELL: Alberts et al., VII edition, Garland Science.