MEDICINE AND SURGERY

Course: THE CELL: MOLECULES AND PROCESSES (CMP)

Year: 1st

Period: Annual

Credits: 19

Objectives

CMP is an integrated course that provides the bases for understanding the relationship between molecular and cell biology, biochemistry, genetics, genomics, and medicine. The course will particularly emphasize the comprehension of biological function at the molecular level, and will provide the foundations of biochemistry and medical genetics, which will be further developed during the second year.

The “Molecular and cellular biology” modules will be focused on understanding structure, function, and turnover of macromolecules, structure and regulation of genes and genomes, as well as cellular compartmentalization, dynamics, and communications. The module will also emphasize the fundamental cellular processes controlling cell growth, division, differentiation, response to stimuli, and death, as well as their relationship with cancer. The importance of recombinant DNA technologies in basic and applied biomedical research will also be illustrated.

The “Biochemistry” module is a compelling synopsis of the major aspects of biochemistry. Fundamental molecular processes are presented and critically discussed in the light of their roles in the human body. Students are provided with key information on how cells and tissues organize the metabolic pathways. This is a pre-requisite to a comprehensive understanding of

the body’s response to stress and pathological conditions. Structure/function relationships of macromolecules are addressed through presentation of their hierarchical structure and discussion of their assembly into functional complexes mediating specific biological processes. Major emphasis is on the regulatory mechanisms that allow energy to be harvested from food, converted into suitable “currency” for utilization in the human body, and

smartly released to fuel biochemical reactions in the cell.

The “Human and medical genetics” module will start with a review of the basic concepts of human genetics, and of the classical and modern approaches to study chromosome structure.

The student will understand the types and extend of genetic variation seen in the human genome, and how this variation affects disease susceptibility. The module will particularly

emphasize how molecular and population genetics have impacted on our understanding of the mechanisms of human disease, providing us with novel diagnostic and therapeutic strategies.

The interdisciplinary aspect of the CMP course is also highlighted by the “Genomic medicine”

nested nanocourse, which will integrate molecular biology, molecular genetics, and medical genetics to give to students the required background to understand physiologic andpathologic mechanisms under an omics perspective. This nanocourse will specifically cover concepts and techniques for the study of the human genome, transcriptome, and proteome, and will include an introduction to the main bioinformatics tools necessary for the genome interpretation in the view of personalized medicine.

Prerequisites

To be allowed to the CMP exam students must have passed the exam of “Principles of the living matter”.

Contents

1st semester

1. Opening lecture

Topics: Presentation of the course and lecturers. Practical infos (textbooks, exam).

2. The scientific method

Module: Cytology, Molecular and Cellular Biology

3. Structure and function of human cells (P. Allavena)

Topics: Cell membrane and cytoplasmic organelles. The fluid-mosaic model of the cell plasma membrane. Main structural features and functions of membranes. Principles oftransmembrane transport.

4: Structure and function of human cells (P. Allavena)

Topics: Vesicle trafficking; Exocytosis and endocytosis; Lysosomes; Peroxisomes; Mitochondria.

5: Structure and function of human cells (P. Allavena)

Topics: The cellular cytoskeleton

6 Structure and function of human cells

Topics: The nucleus and the cell cycle

7. The origin of life

Topics: The biological importance of water. Prokaryotic and eukaryotic cell organization. From prokaryotic to eukaryotic cells: the endosymbiotic theory.

8. FLIPPED Classroom- How does evolution work?

Topics: Darwin, Wallace and the origin of species (Individual work at home, following the instructions on the LMS). Classroom group activity for activation of knowledge.

9. Evolution and medicine

Topics: Example of application of the evolution theory in medicine.

10. The nature of the genetic material

Topics: DNA as the genetic material. Structure of the genetic material, physicochemical properties of DNA molecules.

11-12. Structural and functional properties of DNA molecules

13. Mitosis

Topics: Overview on mechanisms of cell division. Chromosome behavior during cell division in somatic cells. Role of cytoskeleton during mitosis.

14. Meiosis

15. Protein structure

Topics: the central dogma of molecular biology, protein classification, protein constituents, the peptide bond and its properties, the four levels of protein structure, protein function regulation

16. Protein folding

Topics: How proteins acquire their final conformation and their functional properties after synthesis.

17. Chromatin and chromosomes

Topics: The organization of DNA in the cell nucleus, chromatin structure, histones and histone modifications.

18. Main classes of enzymes working on DNA

Topics: Enzymes hydrolyzing the ribose phosphate backbone, synthesizing phosphodiester bonds, modifying DNA topology and modifying nucleic acids bases

19. The mechanism of DNA replication

Topics: DNA Replication: Basic mechanism & enzymology. Semi-discontinuous replication, replication strategies, prokaryotic and eukaryotic DNA polymerases, priming. Origins of replication. The problem of the replication of chromosome ends

20. RNA transcription and metabolism: the transcriptome

Topics: How information flows through macromolecules in the cell. Eukaryotic transcription: RNA polymerases, promoters & enhancers. Eukaryotic transcription factors - general & specific. Complexity of the transcriptome.

21. RNA processing/Alternative splicing in physiology and pathology

Topics: What is RNA splicing and why it has evolved and spread in higher eukaryote genomes. How alternative splicing generates protein diversity. RNA splicing regulation.

22. Meet the expert: Functional Importance of Alternative Splicing in health and disease

Topics: Global approaches to the study of alternative splicing, Splicing correction as a strategy for post-transcriptional gene therapy.

23. The genetic code

Topics: How can 4 nucleotides specify for 20 amino acids. The main properties of the genetic code. How mutations can affect the protein product of a nucleotide sequence. How protein synthesis takes place in the cell?

24. How genes are inherited

Topics: The genetic significance of meiosis. Extensions to Mendel's laws (incomplete dominance, co-dominance, multiple alleles), ABO groups. Penetrance, expressivity, pleiotropy.

25. Cytogenetics and clinical cytogenetics

Topics: Preparation and interpretation of the karyotype. Genomic and chromosomal abnormalities and their importance in the phenotype and reproductive risk. Overview of the main techniques (classical karyotyping, FISH -Fluorescence in Situ Hybridization- and CGH Comparative Genomic Hybridization-arrays).

26. Genetic variation

Topics: Polymorphisms and mutations: classification and functional consequences.

27-28. Protein sorting and secretory pathway

Topics: Post-translational modifications and fate of proteins after synthesis. Signals and mechanisms of protein sorting to organelles and secretory pathway.

29-30-31. Cell signaling

Topics: General principles of cell signaling. Signal transduction. Nuclear receptors. G-protein coupled receptors. Enzyme-coupled receptors. Second messengers. Protein kinases and molecular switches.

Module: Biochemistry

32. How chemical reactions take place in the cell

Topics: Principles of the metabolic control. Metabolic functions as complex systems. Enzymes and cofactors. Mechanisms and control of the enzymes' activity. Thermodynamics of metabolism.

33. Biochemistry in the lab 1

Topics: Protein purification, analysis and sequencing.

2nd semester

Module: Molecular and Cellular Biology

1. The structure of eukaryotic genes

Topics: What is a gene? Structure of prokaryotic and eukaryotic genes. Main features of eukaryotic genes.

2. The mutability of the genome

Topics: Causes of mutation: spontaneous and induced. Agents that induce mutation. Common mutations due to DNA replication.

3. DNA repair mechanisms

4. Recombinant DNA

Topics: What is rDNA? Tools of rDNA technology, making a recombinant DNA molecule, DNAprobes and hybridization, main applications of rDNA.

5. Polymerase Chain Reaction (PCR) & DNA sequencing

Topics: The discovery of PCR, the principles of a PCR reaction, what can you do with PCR? Sanger sequencing.

6. Applications of recombinant DNA technology

Topics: Overview on the main approaches used to produce recombinant proteins of medical relevance by DNA recombinant methods.

7. The regulation of gene expression

Topics: How cells modulate gene expression. Levels of gene expression regulation: from chemical modification of DNA to post-transcriptional and post-translational regulation. The principles of DNA-protein interaction.

8. The regulation of gene expression – part 2

Topics: Regulation of gene expression in prokaryotes. Anabolic and catabolic operons. The transcriptional regulation of gene expression in eukaryotes. Enhancers, silencers and insulators. The epigenetic regulation of gene expression.

9. The regulation of gene expression - part 3

Topics: Post-transcriptional regulation of gene expression. Attenuation. Alternative splicing, differential polyadenylation, RNA editing. Control of mRNA localization and stability. RNA interference.

10-11. Noncoding RNAs part 1 and 2

Topics: The non-coding RNA revolution. Overview on the main classes of non-coding RNAs. Post-transcriptional regulation by small RNAs in prokaryotic and eukaryotic organisms. Longnon-coding RNAs: structural features and mechanism of action. Competing endogenous RNAs.

12. Forensic science and Paternity testing

Topics: Forensic science: kind of traces, DNA fingerprinting. Paternity testing.

13. Cell cycle regulation

Topics: Cell cycle phases. Regulation of cell cycle by extracellular stimuli. Cell cycle checkpoints. Role and regulation of cyclins.

14. Apoptosis

Topics: Differences between necrosis and apoptosis. Roles of apoptosis. Molecular regulation of apoptosis. Intrinsic and extrinsic apoptotic pathways. Caspases. The apoptosome. The Bcl-2 family.

15-16. Oncogenes and cancer

Topics: General facts and stats about cancer. Cancer as a disease of cell behavior. Hallmarks of cancer. In vitro contact inhibition and in vivo tumor progression. Control of cell proliferation in cancer. Isolation of the first human oncogene. Classes of oncogenes. Mechanisms of proto-oncogenes activation. Chromosomal alterations and cancer. Multiple mutations in cancer progression. Tumor suppressor genes.

17. Viruses

Topics: Classification of viruses: DNA and RNA viruses. Structure of viral particles. Viral tropism. The cycle and the genome organization of retroviruses. Role in human disease. Use of viral vectors for gene therapy.

18. Meet the expert: Stem cells

Topics: Properties and sources of stem cells. Induced pluripotent stem cells. Cell fate determination. Cancer stem cells. Application of stem cells in therapy.

19. Genome editing by CRISPR/CAS: medical opportunities and ethical dilemma

Topics: Genome Engineering with CRISPR-Cas9, a breakthrough technology. The case of He Jiankui and the first CRISPR-edited babies. (Individual work at home, following the instructions on the LMS). Classroom activity and discussion for activation of knowledge.

Module: Human and Medical Genetics

20. Mendelian pedigree patterns and their complications

Topics: How to build up a pedigree. Examples of autosomal dominant, autosomal recessive, X-linked, Y-linked and mitochondrial inheritance. Incomplete penetrance, expressivity, male lethality, de-novo mutations, mosaicism, phenocopies, complementation, and mitochondrial

21. Genetic mapping of Mendelian traits

Topics: polymorphisms as a tool for genetic mapping, recombinants and non-recombinants, two-point and multi-point mapping, the concept of LOD score.

22. Prototypic Mendelian diseases: cystic fibrosis and thalassemia

Topics: The quest for the gene causing cystic fibrosis. The organization of the globin loci. Sickle cell anemia. Alpha and beta thalassemia

23. Dynamic mutations

Topics: The concept of microsatellite instability. Trinucleotide repeat expansion disorders and their classification. Anticipation. Fragile X syndrome, Huntington disease, myotonic dystrophy.

24. Population genetics

Topics: Allele frequencies in populations. Hardy-Weinberg equilibrium. Genetic drift, population bottlenecks and founder effects. Genetic selection. The concept of the heterozygote advantage.

25. Genetics of complex (multifactorial) diseases

Topics: The polygenic and multifactorial nature of common diseases. Estimating the contribution of genetic and environmental factors. Linkage disequilibrium. Principle of allelic association.

Module: Biochemistry

26. Catabolic pathways of carbohydrate metabolism

Topics: Carbohydrate digestion. Glycolysis. Catabolism of fructose, galactose and mannose. Homolactic fermentation. The pentose phosphate pathway. Glycogen phosphorylase.

27. Anabolic pathways of carbohydrate metabolism

Topics: Properties and functions of glycogen synthase. Gluconeogenesis.

28. Acetyl-CoA and the citric acid cycle

Topics: Acetyl-CoA as a metabolic “hub” at the crossroad of anabolic and catabolic pathways. Acetyl-CoA fuels the citric acid cycle. Reactions and regulation of the citric acid cycle.

29. Mitochondrial ATP synthesis

Topics: Oxidation-reduction reactions in the mitochondrial redox centers. Proton gradient across the inner mitochondrial membrane and ATP synthesis (oxidative phosphorylation).

30. Lipid metabolism

Topics: Metabolism of fatty acids and eicosanoids. Biosynthesis of triacyglycerols and membrane phospholipids. Cholesterol and bile acids metabolism.

31. Synthesis and degradation of amino acids

Topics: Nitrogen homeostasis. Digestion and absorption of proteins. Urea cycle. Synthesis and breakdown of amino acids.

32. Heme metabolism

Topics: Synthesis of δ-aminolevulinic acid and porphobilinogen. Uroporphyrinogen IIIsynthase. Bilirubin and its conjugation with glucuronic acid. Ferrochelatase. Iron absorption and transport.

33. An integrated view of fuel metabolism

Topics: Organ specialization. Insulin, glucagon and catecholamines. Regulation of energy metabolism, appetite and body weight. Alterations of the metabolic homeostasis.

34. Nucleotide synthesis and degradation

Topics: *De novo* and salvage pathways. Ribonucleotide reductase. Synthesis of dTTP. Nucleotide catabolism.

35. Biochemistry in the lab 2

Topics: Monoclonal antibodies.

Module: Nanocourse of Genomic Medicine

This module is intended to provide the students cutting-edge information on the most advanced approaches to the analysis of the human genome and their application to the understanding of human disease pathogenesis and predisposition to multifactorial disorders.

36. Human genome organization

Nuclear and mitochondrial genomes. The C-value paradox. Classes of repetitive DNAelements. Gene families. Organization of repetitive elements in the human genome. Segmental duplications.

37. How to analyze the genome

DNA sequencing from Sanger to today. Next-generation sequencing (NGS) 2nd and 3rd generation DNA sequencing. Short-read and long-read approaches.

38-39. NGS for the identification of the molecular basis of Mendelian diseases. From genome sequence to the causative variant

From candidate gene analysis to whole genome sequencing. Targeted resequencing vs whole genome sequencing. Exome sequencing: flowchart and examples on how to design a study. The big challenge to fish out the pathogenic variants. How to find a novel disease gene: from theory to practice.

40. Application of NGS to study the transcriptome

Applications of NGS technologies in biomedicine: not only detection of genetic variants. Detection of cell-free DNA. Metagenomics. Transcriptomics: techniques and applications.

41. Genome-wide approaches to complex diseases

Genome-wide association studies. Missing heritability. Burden of rare variants.

42. What we have learned from omics approaches

LD structure of human genome. Genomic consortia, Databases. From phenotypes to mutations, to mutations without a phenotype: redefining the classical concept of Mendelian diseases.

43. Metabolomics

Metabolomics as a branch of systems biology. From targeted analyses to holistic approaches.Preparative and analytical tools of metabolomics. Applications of metabolomics in the discovery of cancer biomarkers and new unexpected metabolic links.

Teaching Methods

Fronctal lectures and flippled classes.

Verification of learning

Students' evaluation will be assessed through a written examination with multiple choice questions. The exam will include 66 questions, proportionally distributed among the different modules. Each question will score 0.5 point; no penalties will be applied for wrong answers. The threshold score for passing the exam will be 18 points. At least 40% of questions of each module must be correct to pass the test.

There will be also an intermediate test at the end of the first semester (February). The test will be composed of 30 questions (multiple choice, each question will score 1 point, maximum30/30). The intermediate test is not mandatory. Students who will not take (or will not pass) the intermediate test will have to take it together with the final exam. Students have 1 month after the test to reject the result in case they are not satisfied by the grade, after that if the grade is ≥18/30 it will be considered as accepted. Students who passed the intermediate test will be able to take a II semester test consisting of 36 questions at the end of the second semester, to complete the exam. This option can be seized only till the September session, after that students will have to pass the full 66 question exam. Additional details on the

intermediate test and on the final exam can be found in the slides of the course introductory lesson.

Texts

- Alberts B et al. Molecular biology of the cell. 6th edition. Garland Science, 2014.
- Watson J et al. Molecular biology of the gene. 7th edition. Pearson, 2013.
- Thompson & Thompson. Genetics in medicine. 8th edition. Saunders, 2015.
- Principles of Biochemistry. International student version - Voet, Voet and Pratt - Publisher: Wiley (4th edition) *or* Principles of Biochemistry. Global edition- Voet, Voet and Pratt - Publisher: Wiley
- Lehninger Principles of Biochemistry - Albert Lehninger, David L Nelson and Michael M Cox - Publisher: W. H. Freeman (7th edition)
- Biochemistry – Berg JM, Tymoczko JL and Stryer L - Publisher: Palgrave MacMillan (7th edition)
- Genetics in Medicine - Thompson & Thompson - Publisher: TElsevier (8th edition)