

Course title Epigenetics

Department Department of biology

School of Medicine University of Zagreb

Address Šalata 3, 10 000 Zagreb

Total ECTS points 3

Course leader Professor Ljiljana Šerman, MD PhD

Course associates Professor Tamara Nikuševa Martić; Professor Frane Paić; Valentina Karin-Kujundžić, PhD.

Teaching plan

	No. classes
Lecture	2
Seminar	7
Practical	6
Total	15

1 class = 45 minutes

Course description

The course offers students the basic knowledge of epigenetic mechanisms and the resulting consequences of their changes effecting the cells. Compared to normal tissues, there are a number of clinical entities that show changes in DNA methylation status, in the histone code or in the small interfering RNA expression. Particular emphasis will be placed on the role of epigenetic changes in etiopathogenesis of oral cancers. At the same time, students will be supplied with information on the possibility of using epigenetic markers for diagnostic and therapeutic purposes. The diagnostic use of epigenetic changes in free DNA will be covered as well. The benefits of using the free serum DNA analysis for early tumor diagnosis and for prognosis follow up will be explained. Because epigenetics today occupies a significant domain in biomedicine and encompasses vast areas engaging a large number of scientists, this course will facilitate understanding of the basis of epigenetics and epigenetic changes in health and disease.

The proposed educational themes are specifically selected to enable students to study the problem on the specific cases from the original scientific research carried on for years in our laboratory. The practical part of the proposed course will enable students to detect epigenetic changes in healthy and tumor tissues, with emphasis on oral cancer samples.

Students will be offered insights into the most recent research advances in this scientific domain. The emphasis will be placed on the understanding of epigenetic changes in healthy and pathological tumorous head and neck tissues. Specific course topics will be covered in lectures (“Mechanisms of epigenetic regulation of gene expression”), seminars (“Epigenetic changes in the free serum DNA”; „Epigenetic changes in saliva DNA“ and “Epigenetics of tumorous processes”) and in laboratory work (“Analysis of DNA methylation: “CRED-RA method for analysis of DNA methylation” and “MSP – methylation specific PCR”; “Analysis of gene expression at the level of RNA and protein products”).

Learning outcomes

1. To explain the theoretical basis of formation of epigenetic changes
2. To assess and compare the results of the research obtained by genetic and epigenetic analysis
3. To assess the possibility of applying the free circulating DNA analysis and compare the molecular methods used in such analysis, from genetic to epigenetic ones
4. To integrate the analysis of epigenetic changes in understanding of etiopathogenesis of tumors
5. To distinguish and recognize the advantages and disadvantages of using non-invasive methods in early diagnosis and follow-up monitoring of tumors

Course content

Lecture

	Lecture topics	Number of classes/hours
1.	Mechanisms of epigenetic regulation of gene expression	1
2.	Epigenetics: new frontiers in dentistry	1

1 class = 45 minutes

Seminari

	Seminar topics	Number of classes/hours
1.	Epigenetic changes in healthy tissues and in tumors	1
2.	The difference in DNA methylation between a healthy tissue and a tumor	1
3.	Epigenetic changes in free serum DNA	1
4.	Monitoring of epigenetic changes in DNA from saliva – new diagnostic approaches	1
5.	Invasiveness and epigenetics	1
6.	Small interfering RNA (siRNA) molecules and their effect on gene expression	1
7.	Epigenetic drugs	1

1 class = 45 minutes

Vježbe

	practicals topics	Number of classes/hours
1.	DNA isolation	1
2.	Methods of DNA methylation analysis	1
3.	MSP – methylation specific PCR - I	1
4.	MSP – methylation specific PCR - II	1
5.	Basic principles of qPCR	1
6.	Western blotting	1

1 class = 45 minutes

Literature

1. Trygve Tollefsbol: Handbook of Epigenetics: The New Molecular and Medical Genetics, 2nd Edition; Hardcover ISBN: 9780128053881, eBook ISBN: 9780128054772, Elsevier, 2017.
2. Viet CT, Zhang X, Xu K, Yu G, Asam K, Thomas CM, Callahan NF, Doan C, Walker PC, Nguyen K, Kidd SC, Lee SC, Grandhi A, Allen CT, Young S, Melville JC, Shum JW, Viet DT, Herford AS, Roden DF, Gonzalez ML, Zhong JF, Aouizerat BE. Brush swab as a noninvasive surrogate for tissue biopsies in epigenomic profiling of oral cancer. *Biomark Res.* 2021 Dec 20;9(1):90. doi: 10.1186/s40364-021-00349-x.
3. Kumar G, Jena S, Jnaneswar A, Jha K, Suresan V, Singh A. Advancements in diagnostic techniques for oral cancer detection. *Minerva Dent Oral Sci.* 2021 Dec 1. doi: 10.23736/S2724-6329.21.04637-4. Online ahead of print. PMID: 34851069
4. Glibo M, Serman A, Karin-Kujundzic V, Bekavac Vlatkovic I, Miskovic B, Vranic S, Serman L. The role of glycogen synthase kinase 3 (GSK3) in cancer with emphasis on ovarian cancer development and progression: A comprehensive review. *Bosn J Basic Med Sci.* 2021 Feb 1;21(1):5-18. doi: 10.17305/bjbms.2020.5036.
5. Karin-Kujundzic V, Kardum V, Sola IM, Paic F, Skrtic A, Skenderi F, Serman A, Nikuseva-Martic T, Vranic S, Serman L. Dishevelled family proteins in serous ovarian carcinomas: a clinicopathologic and molecular study. *APMIS.* 2020 Mar;128(3):201-210. doi: 10.1111/apm.13012.
6. Karin-Kujundzic V, Sola IM, Predavec N, Potkonjak A, Somen E, Mioc P, Serman A, Vranic S, Serman L. Novel Epigenetic Biomarkers in Pregnancy-Related Disorders and Cancers. *Cells.* 2019 Nov 18;8(11):1459. doi: 10.3390/cells8111459.

Additional reading

1. Silva LC, Borgato GB, Wagner VP, Martins MD, Rocha GZ, Lopes MA, Santos-Silva AR, de Castro Júnior G, Kowalski LP, Nor JE, Squarize CH, Castilho RM, Vargas PA. Cephaeline is an inducer of histone H3 acetylation and inhibitor of mucoepidermoid carcinoma cancer stem cells. *J Oral Pathol Med.* 2021 Oct 18. doi: 10.1111/jop.13252.
2. Mehterov N, Vladimirov B, Sacconi A, Pulito C, Rucinski M, Blandino G, Sarafian V. Salivary miR-30c-5p as Potential Biomarker for Detection of Oral Squamous Cell Carcinoma. *Biomedicines.* 2021 Aug 24;9(9):1079. doi: 10.3390/biomedicines9091079.
3. Sobocan N, Katusic Bojanac A, Sincic N, Himelreich-Peric M, Krasic J, Majic Z, Juric-Lekic G, Serman L, Vlahovic M, Jezek D, Bulic-Jakus F. A Free Radical Scavenger Ameliorates Teratogenic Activity of a DNA Hypomethylating Hematological Therapeutic. *Stem Cells Dev.* doi: 10.1089/scd.2018.0194.
4. Kafka A, Karin-Kujundžić V, Šerman L, Bukovac A, Njirić N, Jakovčević A, Pećina-Šlaus N. Hypermethylation of Secreted Frizzled Related Protein 1 gene promoter in different astrocytoma grades. *Croat Med J.* 2018 Oct 31;59(5):213-223.
5. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S, Serman L. The role of the Hedgehog signaling pathway in cancer: A comprehensive review. *Bosn J Basic Med Sci.* 18: 8-20, 2018
6. Kardum V, Karin V, Glibo M, Skrtic A, Martic TN, Ibisevic N, Vranic S, Serman L. Methylation-associated silencing of SFRP1 gene in high-grade serous ovarian carcinomas. *Ann Diagn Pathol* 31:45-49, 2017

CV (*curriculum vitae*) and bibliography of course leader

Ljiljana Šerman was born on November 7, 1967 in Zagreb. She graduated from the School of Medicine, University of Zagreb in 1992. She completed her medical internship at the Clinical Hospital

“Sestre milosrdnice” from 1992 to 1993, after which she has a valid medical license (Croatian Medical Chamber). Since 1995, she has been working as an assistant at the Department of Biology, School of Medicine, University of Zagreb, where in 1999 she received her master's degree, and in 2005 she received her PhD. In 2007 she became an assistant professor, in 2012 an associate professor, and in 2018 a full time professor at the Department of Biology, School of Medicine, University of Zagreb. She specialized in glycobiology at the Department of Biochemistry and Molecular Biology of the Faculty of Pharmacy and Biochemistry in Zagreb, and completed courses: "The Art of Medical Teaching" in Zagreb, "Basic course in stereology" in Ljubljana and "Basic and advanced genetic counseling" in Bertinoro. In 2016, she completed a four-year education in Integrative Psychotherapy.

She has participated in 3 domestic, one HRZZ project and 2 international scientific research projects ("The expression of the Hedgehog signaling pathway components Gli1, Gli3 and PTCH1 in invasive apocrine carcinoma of the breast" in collaboration with the University of Qatar), and so far led 5 university grants. She is currently leading the Work Package "Pathology in Pathological Pregnancy" within the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Subunit: Biomedical Research on Reproduction and Development, within the project of the European Structural and Investment Funds, Competitiveness and Cohesion; European Regional Development Fund: Regenerative and Reproductive Medicine - Research on New Platforms and Potentials (KK.01.1.1.01.0008). Her scientific work consists of 54 scientific papers published in internationally peer-reviewed journals. She has presented her works at over 50 international and domestic congresses. Her primary scientific interest is embryogenesis and carcinogenesis of mammals, especially differences in the differentiation of trophoblasts and tumors at the epigenetic level, i.e. differences in the expression of specific proteins during implantation and placentation as well as tumorigenesis with special emphasis on their regulation by DNA methylation. She is the author and co-author of several teaching texts and book chapters. She has received 9 awards and scholarships for her scientific work so far. She participates in undergraduate classes at 8 courses of the School of Medicine and one course of the Faculty of Dentistry, and in postgraduate classes at 6 courses of the School of Medicine and one course of the School of Dental Medicine. She supervised 5 student papers that were awarded with the Rector's and Dean's Prize, 18 final and graduate theses and 5 doctoral dissertations. At the School of Medicine, she participates in the work of the Committee for diploma theses, final exam and diploma exam, the Committee for teaching, the Committee for scientific work of students and the Committee for enrollment in the first semester and test exams. She actively participates in the popularization of science as part of the "Science Festival", and in workshops at the Department of Biology of the School of Medicine, and has written over 20 popular scientific texts for Liječničke novine. She was a part of the editorial team that published the manual: Croatian Guidelines for Genetic Counseling and Testing for Hereditary Breast and Ovarian Cancer (ed: Brkljačić, Dedić-Plavetić, Haller, Levanat, Podolski, Šerman, Vrdoljak, Vukota, Žic, Žigman), Medicinska naklada, Zagreb 2016. She is one of the authors of the electronic manual: Ask everything - Everything for her, 2016, intended for women with breast cancer, and in 2014 and 2015 she volunteered as a psychotherapist-educator in psychological and genetic counseling for women with from cancer as well as members of their families. She has given a number of scientific and professional lectures, most of them dedicated to genetic counseling. In 2020, she translated the ESMO manual Personalized Medicine into Croatian. Since 2014 she has been a member of the Editorial Board of the international journal *Bosnian Journal of Basic Medical Sciences*, and since 2016 a member of the Executive Board of the Croatian Clinical Genetics Society.

<https://www.bib.irb.hr/pregled/profil/27622>

Selected publications:

1. Sremac M, Paic F, Grubelic Ravic K, Serman L, Pavicic Dujmovic A, Brcic I, Krznaric Z, Nikuseva Martic T. Aberrant expression of SFRP1, SFRP3, DVL2 and DVL3 Wnt signaling pathway components in diffuse gastric carcinoma. *Oncol Lett.* 2021 Dec;22(6):822. doi: 10.3892/ol.2021.13083.
2. Vrhovac Madunić I, Karin-Kujundžić V, Madunić J, Šola IM, Šerman L. Endometrial Glucose Transporters in Health and Disease. *Front Cell Dev Biol.* 2021 Sep 6;9:703671. doi: 10.3389/fcell.2021.703671.
3. Sola IM, Serman A, Karin-Kujundzic V, Paic F, Skrtic A, Slatina P, Kakarigi L, Vranic S, Serman L. Dishevelled family proteins (DVL1-3) expression in intrauterine growth restriction (IUGR) placentas. *Bosn J Basic Med Sci.* 2021 Aug 1;21(4):447-453. doi: 10.17305/bjbms.2020.5422.
4. Žigman T, Lukša I, Mihaljević G, Žarković M, Kirac I, Vrdoljak DV, Šerman L. Defining health-related quality of life in localized and advanced stages of breast cancer - the first step towards hereditary cancer genetic counseling. *Acta Clin Croat.* 2020 Jun;59(2):209-215. doi: 10.20471/acc.2020.59.02.02.
5. Glibo M, Serman A, Karin-Kujundzic V, Bekavac Vlatkovic I, Miskovic B, Vranic S, Serman L. The role of glycogen synthase kinase 3 (GSK3) in cancer with emphasis on ovarian cancer development and progression: A comprehensive review. *Bosn J Basic Med Sci.* 2021 Feb 1;21(1):5-18. doi: 10.17305/bjbms.2020.5036.
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10. Katusic Bojanac A, Rogosic S, Sincic N, Juric-Lekic G, Vlahovic M, Serman L, Jezek D, Bulic-Jakus F. Influence of hyperthermal regimes on experimental teratoma development in vitro. *Int J Exp Pathol.* 2018; 99(3):131-144.
11. Zmijanac Partl J, Karin V, Skrtic A, Nikuseva-Martic T, Serman A, Mlinarec J, Curkovic-Perica M, Vranic S, Serman L. Negative regulators of Wnt signaling pathway SFRP1 and SFRP3 expression in preterm and term pathologic placentas. *J Matern Fetal Neonatal Med* 31: 2971-79, 2018
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14. Jurkovic I, Gecek I, Skrtic A, Zmijanac Partl J, Nikuseva Martic T, Serman A, Galesic Ljubanovic D, Serman L. ELF5 transcription factor expression during gestation in humans and rats—an immunohistochemical analysis. *J Matern Fetal Neonatal Med* 30: 1261-66, 2017
15. Vrbanović Mijatović, V., Šerman, L., Gamulin, O. Analysis of pulmonary surfactant by Fourier transform infrared spectroscopy after exposure to sevoflurane and isoflurane. *Bosn J Basic Med Sci* 17: 38-46, 2017
16. Borovečki, A., Braš, M., Brkljačić, B., Serman L, (...), Žic, R., Žigman, T. Guidelines for genetic counselling and testing for hereditary breast and ovarian cancer | [Smjernice za genetičko savjetovanje i testiranje na nasljedni rak dojke i jajnika] 2017 Lijecnicki Vjesnik
17. Fabijanovic D, Zunic I, Martic TN, Skenderi F, Serman L, Vranic S. The expression of SFRP1, SFRP3, DVL1, and DVL2 proteins in testicular germ cell tumors. *APMIS*. 124:942-949, 2016
18. Serman L, Zunic I, Vrsaljko N, Grbesa D, Gjurcevic E, Matasin Z, Nikuseva Martic T, Bulic Jakus F, Tlak Gajger I, Serman A: Structural changes in the rat placenta during the last third of gestation discovered by stereology, *Bosn J Basic Med Sci* 15: 21-5, 2015
19. Partl JZ, Fabijanovic D, Skrtic A, Vranic S, Martic TN, Serman L: Immunohistochemical Expression of SFRP1 and SFRP3 Proteins in Normal and Malignant Reproductive Tissues of Rats and Humans, *Appl Immunohistochem Mol Morphol* 22: 681-7, 2014
20. Serman L, Nikuseva Martic T, Vranic S: T-cell factor 1 expression in germ cell tumors with trophoblastic differentiation, *Pathol Int* 64: 86-7, 2014
21. Serman L, Nikuseva Martic T, Serman A, Vranic S. Epigenetic alterations of the Wnt signaling pathway in cancer: a mini review, *Bosn J Basic Med Sci* 14: 191-4, 2014
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24. Muzic V, Katusic Bojanac A, Juric-Lekic G, Himmelreich M, Tupek K, Serman L, Marn N, Sincic N, Vlahovic M, Bulic-Jakus F: Epigenetic drug 5-azacytidine impairs proliferation of rat limb buds in an organotypic model-system in vitro, *Croat Med J* 54: 489-95, 2013