

UNDER THE AUSPICES OF

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Ministry of Science, Education and Sports of the Republic of Croatia - MZOS

Institute for Clinical Medical Research of Clinical Hospital Center Sestre Milosrdnice, Zagreb

City of Zagreb

Ljudevit Jurak

26th Ljudevit Jurak

International Symposium
on Comparative Pathology

JUNE 2, 2017, PALACE HOTEL ZAGREB

J.J. Strossmayer Square 10, Zagreb, Croatia



FINAL PROGRAMME & BOOK OF ABSTRACTS

Dear colleagues,

The Ljudevit Jurak International Symposium on Comparative Pathology has a history of international collaboration and involves a wide array of professionals within the field of pathology. What separates and defines this symposium is its collaboration with veterinary pathologists, and furthermore, comparison of pathologic changes in human and veterinary medicine. With this symposium we honor Professor Ljudevit Jurak, who founded the first pathology department in Croatia, in Clinical Hospital Center Sestre milosrdnice. Professor Ljudevit Jurak contributed greatly to Veterinary and Human Pathology as well as Forensic Medicine and represents an historical and eminent figure in the field of pathology both in Croatia and worldwide.

The main topic of this 26th Symposium is MOLECULAR PATHOLOGY OF SOLID TUMORS. Invited lecturers will present their topics on the most relevant fields within molecular pathology. All pathologists dealing with Human and Veterinary Pathology as well as Forensic Pathologists are encouraged to participate and support this multidisciplinary meeting.

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Friday June 2, 2017.

08:00 Registration

08:30 Opening ceremony

Chairpersons: Božo Krušlin, Gregor Mikuz

09:00 George J. Netto (USA): Personalized management in bladder cancer:
The promise of novel molecular taxonomy

DISCUSSION

09:40 Semir Vranić (BIH): Recent advances in molecular pathology
of breast cancer

DISCUSSION

10:20 Coffee break

Chairpersons: Marina Kos, Snježana Tomić

10:40 Zoran Gatalica (USA): Colorectal carcinoma: a brief review of predictive
biomarkers in the era of personalized medicine

DISCUSSION

11:20 Sven Seiwerth (CRO): Molecular diagnostics in lung pathology

DISCUSSION

12:00 Fabio Del Piero (USA): Molecular pathology of solid tumors in
domestic animals

DISCUSSION

12:40 General Assembly of Croatian Society of Pathology and Forensic Medicine

13:00 Lunch

Chairpersons: Zoran Gatalica, Snježana Dotlić

14:00 George J. Netto (USA): A bullish outlook on the future of anatomic pathology in the era of precision medicine

DISCUSSION

14:40 Andrew L. Folpe (USA): The impact of advances in molecular biology on selected aspects of soft tissue pathology (part 1)

15:20 Andrew L. Folpe (USA): The impact of advances in molecular biology on selected aspects of soft tissue pathology (part 2)

DISCUSSION

16:00 Coffee break

Chairpersons: Hrvoje Čupić, Sven Seiwert

16:20 Suzana Tkalčić (USA): Molecular diagnostics, biomedical research and One Health

DISCUSSION

16:50 Zoran Gatalica (USA): Comprehensive tumor profiling for biomarkers of drug response in cancers of unknown primary site.

DISCUSSION

17:30 Molecular diagnostics of solid tumors - current practice in Croatia (Reports from University Centres in Zagreb, Split, Rijeka and Osijek)

18:10 Closing ceremony

20:30 Symposium dinner

We hope that you will enjoy the scientific program as well as the social events. We also hope that you will find some free time to enjoy our beautiful city and its surroundings.

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kape | maske | ogrtači | kombinezoni | rukavci | navlake za cipele |
trljačice | brisači | sterilne prekrivke | sterilne navlake | sterilni setovi

STVARAMO DA ŠTITI!



Lectures

**PERSONALIZED MANAGEMENT IN BLADDER CANCER:
THE PROMISE OF NOVEL MOLECULAR TAXONOMY**

George Jabboure Netto, M.D.

Professor and Chair of Pathology, University of Alabama at Birmingham

Empowered by the recent advances in next generation sequencing and bioinformatics technology, an unprecedented wave of integrated transcriptomic and genomic studies have impacted the field of bladder cancer. These studies not only have confirmed previously charted genetic pathways in bladder cancer development but also have led to the discovery of numerous additional crucial driver genetic alterations. As a result, a novel genomic-based taxonomy is emerging that promises to better define clinically relevant intrinsic subtypes of bladder cancer. The current review is an update on the above advances and their significant implications.

THE IMPACT OF ADVANCES IN MOLECULAR GENETICS ON SELECTED ASPECTS OF SOFT TISSUE PATHOLOGY

Andrew L. Folpe, M.D.

Professor of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Our understanding of the molecular underpinnings of soft tissue neoplasms has exploded over the past 20 years. Indeed, along with hematopathology, soft tissue pathology has made the greatest strides in integrating this new information into all aspects of diagnosis and classification. This lecture will focus on selected areas of soft tissue pathology where advances in molecular genetics have brought clarity to our understanding of soft tissue tumors.

Liposarcoma is perhaps the best example of an area where molecular pathology has changed soft tissue tumor classification. It is now understood that well-differentiated and dedifferentiated liposarcoma represent different forms of a single entity, defined by amplification of genes found on 12q, such as MDM2 and CDK4. Similarly, round cell liposarcoma is now understood to be simply the high-grade form of myxoid liposarcoma, as both share common rearrangements of the DDIT3 gene with FUS or less often EWSR1. It is also now clear that so-called “mixed type” liposarcoma does not exist as a discrete entity, representing instead a variety of unusual morphological manifestations of well-differentiated, myxoid and pleomorphic liposarcoma.

Advances in molecular pathology have also allowed us to definitely recognize the existence of malignant forms of ossifying fibromyxoid tumor, as both typical and malignant OFMT show rearrangements in the PHF1 gene. Similarly, demonstration of MYOD1 and PIKC3A rearrangements has firmly established spindle cell and sclerosing rhabdomyosarcoma as a distinct subtype of rhabdomyosarcoma, rather than simply a variant of embryonal or alveolar rhabdomyosarcoma, as was originally debated. Additionally, improved understanding of molecular pathogenesis has given us powerful diagnostic tools, especially for soft tissue sarcomas that otherwise lack specific markers (e.g. alveolar soft part sarcoma) or show confusing immunohistochemical profiles (e.g. low-grade fibromyxoid sarcoma). Improved understanding of the molecular underpinning of soft tissue tumors has also allowed us to better clarify the relationship (or lack thereof) between morphologically related tumors such as hemosiderotic fibrolipomatous tumor, pleomorphic hyalinizing angiectatic tumor and myxoinflammatory fibroblastic sarcoma, all of which have been reported to show rearrangements involving the TGFBR3 and MGEA5 genes. Finally, certain molecular genetic events, such as PAX&FOXO1A fusion in alveolar rhabdomyosarcoma and PDGFB rearrangement in dermatofibrosarcoma, have been shown to either be predictive of outcome or response to targeted therapy.

This lecture will also discuss a number of limitations in the application of molecular diagnostics to the study of soft tissue tumors, in particular tumors showing marked molecular heterogeneity (e.g. Ewing sarcoma), multiple different tumors showing rearrangements of the same genes (e.g. EWSR1 and FUS), tumors with rare variant fusions not detectable by conventional methods (e.g. SS18L1 rearrangements in synovial sarcoma) and even multiple clinically and pathologically distinct tumors carrying identical gene fusions. The importance of integrating molecular genetic findings into the overall clinicopathological scenario will be stressed.

Key words: sarcoma, molecular genetics, liposarcoma, rhabdomyosarcoma

COLORECTAL CARCINOMA: A BRIEF REVIEW OF PREDICTIVE BIOMARKERS IN THE ERA OF PERSONALIZED MEDICINE

Zoran Gatalica, MD, DSc and Joanne Xiu, PhD

Caris Life Sciences, Phoenix, AZ

Colorectal cancer (CRC) incidence and mortality rates are remarkably high worldwide, with 1.4 million new cases and approximately 700,000 deaths per year. Based on gene expression, four consensus molecular subtypes (CMS) of CRC with distinguishing characteristics have been proposed:

CMS1 (microsatellite instability immune subtype: hypermutated subtype of CRC, microsatellite unstable with a strong immune activation); CMS2 (canonical subtype of CRC: epithelial subtype with upregulation of the WNT and MYC signaling pathways); CMS3 (metabolic subtype of CRC: epithelial subtype with metabolic dysregulation); and CMS4 [mesenchymal subtype of CRC with prominent transforming growth factor- β (TGF- β) activation, stromal invasion and neoangiogenesis. It remains to be seen how this classification would affect clinical practice.

A simplified classification of CRC based on microsatellite DNA instability (MSI) status into two broad subgroups, MSI-high (MSI-H; 15%) and MSI-negative (low or stable; 85%), is presented in an effort to highlight therapeutic differences between these two easily separable groups in pathology practice. MSI-H CRCs respond poorly to 5-FU based chemotherapy (based on thymidylate synthase overexpression), but they may be efficiently treated with camptothecin derivatives (based on topoisomerase 1 overexpression). Due to an active immune microenvironment and high expression of various checkpoint molecules, MSI-H CRCs are good candidates for targeted immunotherapy with immune checkpoint inhibitors.

MOLECULAR DIAGNOSTICS IN LUNG PATHOLOGY

Prof. dr. Sven Seiwert, M.D, Ph.D.

Head of Department of Pathology and Institute of Pathology
University of Zagreb School of Medicine

Molecular diagnostic techniques have recently occupied the most prominent place in a lot of medical fields, among others in lung oncology. The classical clinical approach to lung tumor therapy resulted until recently in a most simplified request to the diagnostic morphologist, dividing malignant epithelial tumors into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Further differentiation of NSCLC was welcome, although basically of no clinical value. In the mid 2000-nds with the appearance of drugs targeting cells with specific mutations the situation dramatically changed. In lung cancer during a few years a genetic spectrum of different cancer types defined by specific mutations evolved. The relation of this molecular changes to morphological types made it necessary also from the clinical aspects to acknowledge the further morphological distinction of NSCLC into squamous cell carcinoma (SCC), not harboring the mutations of interest and non-squamous cell carcinoma (NSCC), including adenocarcinoma and large cell carcinoma. Consequently in 2011 the Joint meeting of IASLC (International Association for the Study of Lung Cancer), ATS (American Thoracic Society) and ERS (European Respiratory Society) yielded a re-classification of the most prominent target group – adenocarcinoma. These changes were mirrored by the two consecutive WHO Blue book editions (2004 and 2015). Currently, according to guidelines adopted by most countries, molecular testing is done for EGFR and ALK mutations, mostly together with ROS testing (for the moment not reimbursed in Croatia), either in the form of reflex testing (ordered by the diagnosing pathologist) or on request (ordered by the clinician). The patients harboring mutations of the respective genes are eligible for TKI or ALK inhibitor therapy (also applicable in ROS mutated cancers). Correct histological classification is important not only in order to lower the testing burden for TKI and ALK inhibitors, but also because the chemotherapy agent pemetrexed is inefficient in SCC and their treatment with VEGF inhibitors (bevacizumab) can produce fatal hemorrhage. The next step already introduced includes immunotherapy (PDL-1 or PD-1 inhibitors) with or without prior testing (opening new perspectives for patients with SCC) as well as other possible molecular targets waiting around the corner. As the described therapy mainly inhibits tumor growth till the period where new, resistant clones take over, reassessment of tumors under therapy by different means (e.g. biopsy or liquid biopsy) also becomes one of the hot topics in pulmonary oncology.

MOLECULAR PATHOLOGY OF SOLID TUMORS IN DOMESTIC ANIMALS

Fabio Del Piero, DVM, PhD, Dipl. ACVP

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Molecular biology and molecular pathology were developed frequently using animal tissues and board certified veterinary pathologists played a large role in the development of these scientific fields and their application in laboratory and domestic animals.

Histologic diagnosis of neoplasms in animals is very often straightforward, but numerous biomarkers are used in veterinary pathology for the immunohistochemical detailed identification of these neoplasms and some have prognostic value, such as proliferation-cell cycle markers and malignancy markers¹.

Urothelial carcinoma², also referred as transitional cell carcinoma (TCC), is the most common urinary bladder neoplasm in the dog, although compared to other cancers it is uncommon. Canine urothelial carcinomas have aneuploidy of several chromosomes. Among these aberrations, high frequency aneuploidy of dog chromosomes CFA 13, 19, and 36 within the same cell is a cytogenetic signature not evident in other canine cancers. In addition, enumeration of CFA 8 in urothelial carcinoma revealed cells to be either diploid ($n = 2$) or tetraploid ($n = 4$). In combination, enumeration of the FISH probes representing regions of these four chromosomes (8, 13, 19, 36) provides an assay that has high specificity and sensitivity when evaluating tumor biopsies. Evaluation of cells collected from urine samples confirms that FISH assay for canine urothelial carcinoma retains a high specificity and sensitivity. Similar approaches are being used to develop additional cytogenetic assays designed to provide diagnostic and prognostic information for a range of other canine cancers, including for example canine leukemia subtypes, mast cell tumors, osteosarcoma, oral melanoma, malignant neural tumors and hemangiosarcoma. In addition, studies examining the cytogenomics of various feline cancers are leading to assays that will provide new tools to aid management of cats diagnosed with injection site sarcoma, gastrointestinal lymphoma/inflammatory bowel disease, and mammary carcinoma.

Amongst the solid tumors, mast cell tumors (MCTs)³ are the most common skin tumors in dogs. They are also the solid domestic animal tumors where molecular ancillary procedures are most commonly used. Their behavior is quite variable and highly dependent on grade (I, II, III). Many are classified as grade II with quite variable behavior within this category. Normal mast cells have transmembrane tyrosine kinase receptors named KIT proteins. MCTs with a mutation of the *c-kit* gene, which encodes KIT protein, specifically internal tandem duplications in exon 11 of the *c-kit* gene, have shown to be more aggressive than those without it. This mutation can be targeted by tyrosine kinase inhibitors. Prognosis of MCTs is currently based on the histologic grading and identification of the mutation in the *c-kit* gene via PCR on biopsies of the mass and in blood and urine.

In addition to the numerous laboratory rodent tumor models of human cancer, there are a few domestic animal solid tumors that are used to study human parallel solid tumors, such as cancer-associated hypercalcemia (canine apocrine anal sac adenocarcinoma), and mechanisms and treatment of bone metastasis (canine prostate adenocarcinoma)⁴.

The cost of the molecular diagnostic procedures sometimes limits the application of these ancillary procedures in the diagnosis of tumors in domestic animals, but there are a progressively increasing number of animal patients benefitting from the application of these techniques. The board certified veterinary pathologist plays a paramount role in the diagnosis, prognosis, recommendation regarding the treatment, and identification of the progression of these conditions.

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RECENT ADVANCES IN MOLECULAR PATHOLOGY OF BREAST CANCER

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Breast cancer is a complex and heterogeneous disease encompassing different histopathologic, molecular genetic and clinical subtypes. Four distinct molecular subtypes have been recognized, namely, luminal A and B cancers, HER2-enriched and triple-negative breast cancer (TNBC).

TNBC is defined by lack of estrogen (ER) and progesterone receptors (PR) and HER2 negativity. TNBC is also heterogeneous and different subgroups have been identified on the basis of protein expression, mRNA, and genomic alterations. Despite all the advances, TNBC still lacks good predictive biomarkers and consequently lacks efficient therapeutic options leading to dismal prognosis. However, next-generation sequencing (NGS) technology has been shown to be a powerful platform to dissect the molecular characteristics of TNBC, offering novel therapeutic targets that are under clinical investigation. In addition, immune checkpoint inhibitors, including PD-1 and PD-L1 proteins, have become potential targets with promising results for the patients with advanced and/or metastatic TNBC. Platinum-based chemotherapy may also be effective for a subset of TNBC harboring DNA repair defects and is being routinely incorporated for the treatment in both neoadjuvant and metastatic settings. Androgen receptor (AR), as the most commonly expressed steroid receptor in TNBC, has also become a potential target for anti-androgen therapy and is actively explored in phase 1-3 clinical trials.

Key words: Breast cancer - molecular pathology - triple-negative breast cancer - biomarkers - targeted therapy

MOLECULAR DIAGNOSTICS, BIOMEDICAL RESEARCH AND ONE HEALTH

Suzana Tkalčić DVM, PhD

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The One Health concept represents a paradigm shift in the western medicine and public health of 21st century that promotes an interdisciplinary and interprofessional approach to health and health-care on a global scale. It promotes a holistic approach, effective communication, resource exchange and respectful collaborations of all stakeholders involved in human health: human medicine, veterinary medicine and environmental health. However, that concept is not foreign to veterinarians around the world, and to many traditional European public health practices, especially ones in the East/Southeast Europe.

As we are facing new health challenges in the modern world, with emerging and re-emerging infections, toxic pollutants, translational research, and biomedical advances, One Health and its comparative medicine stream is a logical paradigm shift to allow for effective and timely reactions to the presenting or predictive problems through the surveillance, biomedical research and capacity building worldwide.

Within the zoonotic infectious diseases and possible pandemics originating from farm animals and poultry farming operations, One Health approach is crucial in understanding the virus biology within the animal host, especially in discovery of genetic signatures that allow cross-species infections, increase in virulence and understanding of the complex biochemical mechanisms that result in the emergence of a pandemic virus. However, rapid disease diagnostics and epidemiological surveillance data inputs warrant complex strategy development across the professional and political borders.

As companion animals age within the socioeconomic platform of the human-animal bond in the modern society, biomedical research in cancer biology and new treatment strategies become readily translational across the species and offer multiple generic and methodological synergies in the areas of biological models in diagnosis and treatment. Novel technologies that include nano-particles, biomarkers, molecular targets and optic sensors are cutting-edge diagnostic techniques still in developing stages that include laboratory animals in pre-clinical phases, but also other mammalian animal models. As they are being tested in animal clinics, the use of companion animal models allows for a faster development of new methods for a rapid diagnostic and therapeutic decision-making.

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COMPREHENSIVE TUMOR PROFILING FOR BIOMARKERS OF DRUG RESPONSE IN CANCERS OF UNKNOWN PRIMARY SITE

Zoran Gatalica, MD, DSc and Joanne Xiu, PhD

Caris Life Sciences, Phoenix, AZ

Cancer of unknown primary (CUP) accounts for approximately 3% of all malignancies. Despite extensive laboratory and imaging efforts, the primary site usually cannot be unequivocally confirmed. Some investigators believe that biologically distinct CUP cases exist. Such cases are thought to have a peculiar and poorly understood biology and a metastasis-causing genetic signature. A cancer of unknown primary site would form when deregulated, premalignant or cancerous stem cells migrated away from their natural tissue and gave rise to a cancer in a new site before or without generating a tumor in their original tissue. For such cases current therapy choices remain non-selective.

Common cancer pathway alterations (Hanahan D and Weinberg RA: Hallmarks of Cancer: The next Generation. Cell 2011;144(5):646-74) comprise biological capabilities acquired during the multistep development of human tumors. They appear across diverse cancer lineages and offer a rational opportunity to treat CUP with specific targeted therapies. Caris Life Sciences had investigated over 2000 patients with CUP and applied multiplatform (DNA and RNA sequencing, protein expression, in-situ hybridizations) to detect biomarkers of drug response. Biomarkers associated with a potential drug benefit were identified in >90% of cases. Associated therapies include different classes of agents including chemotherapeutics, targeted therapy and immune check-point inhibitors.

Sponsors



*Poster
presentation*

POORLY DIFFERENTIATED RENAL METASTASES IN THE GINGIVA OF MANDIBLE AND MAXILLA - CASE REPORT**D. Müller¹, Č. Tomasović-Lončarić¹, D. Mužinić², A. Pačić¹, P. Sesar³, I. Blivajs⁴**¹Department of Pathology, University Hospital Dubrava, Zagreb,²Department of Pathology, University Hospital Merkur, Zagreb,³Department of Pathology, General Hospital "Dr. Ivo Pedišić", Sisak,⁴Department of Maxillofacial Surgery, University Hospital Dubrava, Zagreb, Croatia

An 83-year old female patient was admitted to our Department of Maxillofacial Surgery because of a rapid growing fleshy tumor mass in anterior part of the right mandible. She also had a smaller one, which occurred later during her hospital stay on the gingival surface of the right maxilla. She had been previously operated because of renal carcinoma of clear cell type with sarcomatoid differentiation seven months before admission to our Hospital.

Surgical treatment included total excision of both tumor lesions. We received two excisions with adjacent cortical bone and tumorous tissue, which was histologically poorly differentiated with prominent mitotic activity and nucleoli with areas of bleeding and necrosis, having a sarcomatoid appearance. We performed immunohistochemical analysis of the tumorous tissue, which included positive reaction for CD10 and vimentin and negative reaction for CK(AE1/AE3), Melan-A, HMB45, HHF35, desmin, CK5/6, CK7, EMA, PAX-2 and PAX-8. Due to the IHC profile and morphological characteristics of the tumor we concluded that the tumorous tissue we observed were metastases of the primary renal carcinoma, in this case its sarcomatoid component. Combined with negative PAX-8, having in mind its sensitivity for renal carcinoma, the diagnosis of the renal metastasis can be demanding.

MOLECULAR ALTERATIONS IN PATIENTS WITH LUNG ADENOCARCINOMA. OUR EXPERIENCE OVER A THREE-YEAR INTERVAL (2014-2016)

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Lung cancer is the leading cause of cancer deaths in the world. Nowadays the treatment of lung carcinomas is individualised due to the discovery of targetable genomic alterations. In addition to routine histological examination molecular tests have been used in diagnosis to identify common oncogenic aberrations in those patients, who will respond to targeted therapy.

The aim of our study was to determine the presence and frequency of mutation in EGFR and KRAS genes or ALK gene rearrangement in patients with lung adenocarcinomas diagnosed in our centre over a three-year interval (2014-2016). Additionally, we analysed the clinicopathological differences between patients in each subgroup.

Histopathological data were obtained from the department's computer-based registry. In total 189 patients, 79 females (42%) and 110 (58%) males, were included in our series. Smoking status of each patient was defined as smoker or never-smoker (smoked <100 cigarettes in a lifetime). The majority of them were smokers - 67% of females and 88% of males, respectively. Among 189 cases 23 (12,2%) of adenocarcinomas expressed EGFR mutation, most of the patients were women and never-smokers. In 86 (45,5%) cases KRAS mutations were detected, the majority of the patients were male smokers. From the total number of adenocarcinomas only 5 harboured ALK rearrangements.

In general, smokers were diagnosed at an earlier age comparing to never-smokers. Also, patients diagnosed with adenocarcinoma harbouring KRAS gene mutations were diagnosed younger, especially females. Among 45 deceased patients 25 (55,6%) of them had KRAS gene mutation. Therefore KRAS gene mutation was indirectly confirmed as a poor prognostic factor in our study.

Results associated to patients' characteristics and common genomic aberrations that occur in lung adenocarcinomas fit the data from the literature except for the unusually high rate of KRAS mutations. We attribute this result to our relatively small case series.

**Ki67, ESTROGEN AND ANDROGEN RECEPTORS IN
ORAL SQUAMOUS CELL CARCINOMA - A PILOT STUDY****V. Vučičević Boras¹, Č. Tomasović², D. Gabrić¹, M. Sekerija³, B. Vrdoljak⁴,
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Oral squamous cell carcinoma (OSCC) is a cancer type with a poor survival rate (lethality rate of 48%) and still obscure biology. In a complex tumor microenvironment, estrogen (ER) and androgen receptors (AR) have been suggested to take part in tumor promotion, progression and metastasis. The role of stromal cells in the biology of a tumor is still under investigation. It has been suggested that activated stromal cells within OSCC may produce signal molecules crucial for the development of metastases. Thus, the interaction of key molecules in neoplastic and stromal cells may significantly contribute to the understanding of tumor biology. The aim of the current study was to investigate the association of ER and AR with Ki67 as a biomarker of cell division dynamics in the epithelial and stromal cells of OSCC. In this pilot study 42 male patients were included (mean age 50 years). Immunohistochemistry was performed on paraffin blocks. For each subject 500 epithelial and stromal cells were analysed for ER, AR and Ki67 levels. Results show that ER was not detectable either in the epithelial or in the stromal cells except anecdotally in the salivary glands. The presence of AR positive epithelial cells was significantly associated with AR positive stromal cells ($p=0.01$). Although, not reaching significance due to the low number of subjects, within AR positive epithelial and stromal cells higher Ki67 levels were detected in stromal cells when compared to the AR negative epithelial and stromal cells thus suggesting impact of testosterone on their division rate. The future aim is to include a larger number of patients and other biomarkers in order to achieve a better understanding of the molecular mechanisms that mediate OSCC metastasis and suggest novel therapeutic targets (Funded by CSF No I-1925-2015).

SIGNIFICANCE OF NRAS MUTATIONS IN METASTATIC MELANOMA

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INTRODUCTION: Treatment for metastatic melanoma (MM) still remains a challenge since long-term survival is poor and median overall survival is less than twelve months. Detection of *BRAF* mutation in melanomas implemented molecular targeted therapy. The *BRAF* gene encodes a protein which plays a role in the control of cell proliferation, differentiation, inflammatory responses and apoptosis via the mitogen activated protein kinase (MAPK) pathway. Approximately 40-60% of primary melanomas of sun-exposed skin origin harbor a *BRAF* mutation and those patients are candidates for therapy with BRAF inhibitors. Patients with *BRAF* mutated melanomas have improved response rate and progression-free survival after targeted therapy.

NRAS protein also belongs to a MAPK pathway, but is involved in several other pathways like WNT and PIK3 and its gene mutation results in constitutive activation of those pathways. *NRAS* mutation occurs in 15-20% of primary melanomas, with uncertain significance, where is usually mutually exclusive with *BRAF* mutation. In contrast to *BRAF*, *NRAS* mutations occur in all melanomas of non-veal sites and are rarely present in benign melanocytic nevi.

MATERIAL AND METHOD: A pilot study was conducted on sixteen MM. DNA was isolated from FFPE tissue. BRAF and NRAS mutations were detected by real-time PCR, using Cobas BRAF/NRAS Mutation Test designed to detect 11 mutations of *BRAF* and 25 mutations of *NRAS* gene.

RESULTS: Out of sixteen patients, 75% were male with a median age of 53.5 years and 25% were women with median age of 69.5 years at the time of first diagnosis. BRAF mutation was found in eight cases (50%), seven in male and one in female patients. Eight melanomas were BRAF non-mutated (wild type), but five of them (62.5%) harbor NRAS mutation. Patients with BRAF mutation were younger (median age 46 years) than patients with NRAS mutated MM (median age 60 years). BRAF mutation was more frequently found in metastases from SSM (43%), while *NRAS* was associated with nodular melanoma (40%). We found no difference in clinical characteristics between BRAF and NRAS mutated MM. Only three patients (18.8%) were wild type of both genes.

DISCUSSION: Recent literature reports that the mutant NRAS melanoma has a more aggressive behavior and poorer outcome compared to non-NRAS mutant melanoma. No therapeutic agents have yet been approved for NRAS mutation melanoma. However, NRAS driven melanomas might have few therapeutic options like small molecule MEK inhibitors, small interfering RNAs or immune-based therapy.

CONCLUSION: All BRAF-wild type metastatic melanomas should be additionally tested for NRAS mutations to select patients who also might benefit from targeted therapy.

RESULTS OF THE FIRST YEAR OF RAS MUTATION TESTING IN UNIVERSITY HOSPITAL FOR TUMORS

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INTRODUCTION: Colorectal carcinoma (CRC) is the third most common malignancy worldwide. Survival depends on stage and grade with over 40% of metastatic disease and 5-year overall survival rate of 63%. Treatment options for CRC are surgery, chemotherapy and radiation therapy. The epidermal growth factor receptor (EGFR) was recognized as an important receptor that initiates CRC progression via RAS-RAF-MAPK signaling pathway. Targeted therapy with monoclonal antibodies (cetuximab and panitumumab) have been developed to inhibit EGFR. Anti-EGFR therapy is ineffective in CRC with mutations in *RAS* genes because their proteins function downstream of EGFR-induced cell signaling. *KRAS* gene mutations are detected in 35-54% of CRC, mainly in exon 2 (codons 12 and 13), and *NRAS* mutations are detected in 3-6% of CRCs. Prior anti-EGFR therapy, detection of *KRAS* mutations in exon 2 is obligatory.

MATERIAL AND METHODS: *KRAS* mutations were detected in 90 cases of metastatic CRC. DNA was extracted from FFPE tissue samples and mutations were detected by Cobas® 4800 real-time PCR. In cases without *KRAS* mutation in exon 2, additional *RAS* testing was performed, using several IVD detection kits, designed to detect 28 mutations of *KRAS* and 25 mutations of *NRAS* gene.

RESULTS: Out of 90 patients, 50 patients were male and 40 were female, with a median age of 60 years at the time of first diagnosis. Mutations were detected in 49 cases (54.4%); 47.8% in *KRAS* and 6.6% in *NRAS* gene. *KRAS* gene was mutated in 43 cases; 35 in codon 12/13, 4 in codon 146 and 2 in codons 61 and 117. *NRAS* gene was mutated in 6 cases; 3 in codon 12/13, 1 in codon 61 and 2 in codon 146. In male patients, *KRAS* was mutated in 56% and *NRAS* was mutated in 4% of cases, while in female patients, mutated *KRAS* was detected in 37.5% and *NRAS* was mutated in 10%. Patients with right-sided CRC were older (median age 68 years) and more often with mutations (75%) than patients with tumors localized in the left colon (median age of 58.5 years; 53.5% *RAS* mutations).

DISCUSSION: Recent studies have shown that anti-EGFR treatment is ineffective, not only in patients whose tumors have *KRAS* mutations in exon 2, but also in those patients who have other *RAS* mutations. Moreover, they report that *NRAS*-mutated CRC patients have better prognosis than those with *KRAS* mutations. They have not established the association of clinicopathological characteristics and *RAS* mutations status.

CONCLUSION: Prior to the application of anti-EGFR therapy it is necessary to determine both *KRAS* and *NRAS* mutations status.

CUTANEOUS ROSAI-DORFMAN DISEASE: A CASE REPORT**T. Bota¹, D. Mužinić², D. Müller³, Č. Tomasović-Lončarić³**¹Department of Pathology, General Hospital Virovitica, Virovitica,²Department of Pathology and Cytology, University Hospital Merkur, Zagreb,³Department of Pathology and Cytology, Clinical Hospital Dubrava, Zagreb, Croatia

Rosai-Dorfman disease (RDD) is a rare but distinctive clinicopathologic entity of unknown etiology affecting lymph nodes as well as extranodal sites (43%). In around 3% of patients the disease is limited to the skin. This rare and not well documented form of the disease is known as Cutaneous Rosai-Dorfman disease (CRDD).

CRDD typically occurs in older females and clinically presents in various forms, ranging from single papules to indurated plaques and tumor. It often fails to present any laboratory abnormalities, often progresses benignly and is self-limiting, with no need for more aggressive interventions. The hallmark microscopic signs are mixed inflammatory infiltrate with S-100 positive histiocytes with emperipolesis, the most important feature in a correct diagnosis.

Our case is a 57-year-old female who presented with a one month history of a tumor located on her face. A white indurated area observed on a skin section microscopically corresponded to a well-restricted, dense inflammatory infiltrate of lymphocyte and plasma cells with numerous multinuclear cells and immunohistochemically S-100 and CD68 (KP1) positive histiocytes with visible emperipolesis. At the time of presentation she had no other systemic, extracutaneous or serologic manifestations.

Awareness of the histological aspects present in different lesions, which do not always contain the hallmark microscopic signs of CRDD, is particularly important to correctly diagnose this disorder.

Owing to its favorable outcome and spontaneous resolution, CRDD should not be confused with other benign or malignant lesions such as panniculitis, other cutaneous histiocytoses, xanthogranuloma, malignant histiocytosis, hemophagocytic syndrome, reticulohistiocytoma, sarcoidosis, tuberculosis, dermatofibroma or malignant tumors.

Despite recurrence occurring in a few patients, surgical intervention remains to be the most effective modality of treatment, especially for solitary or localized lesions. A good response to radiotherapy, cryotherapy and chemotherapy has been documented as well as the use of Thalidomide to control extensive cutaneous disease.

IMPACT OF *BRCA* MUTATION ON THE TREATMENT OF OVARIAN CARCINOMA**A. Lukač¹, M. Macan^{1,4}, G. Vujić², I. Babić², V. Matković², M. Mišić³, D. Babić^{1,4}**¹Department of Pathology and Cytology and²Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, University Hospital Centre Zagreb, Zagreb,³Institute of Pathology, School of Medicine, Zagreb,⁴University of Zagreb, School of Medicine, Zagreb, Croatia

The worldwide incidence of ovarian carcinoma is approximately 240 000 cases per year and around 150 000 women die of the disease. 10% of cases are hereditary and 90% of them are sporadic. Some ovarian carcinomas, mostly hereditary, are caused by *BRCA* mutations.

Based on their distinct molecular profile, pathogenesis, histological and immunohistochemical features, currently all ovarian carcinomas are categorized into two groups: low- and high-grade neoplasms. According to this dualistic ovarian carcinoma model, the most common ovarian cancers, serous carcinomas, are also being classified as low- (5%) or high-grade (95%) tumors. These two groups of ovarian serous cancer are presently considered to represent two completely different pathological entities. Low-grade serous carcinomas develop gradually from benign lesions, have a more stable genome with frequent expressions of *KRAS* and *BRAF* mutations and usually behave in an indolent manner. The predominant type, high-grade serous carcinomas, commonly show mutations of p53 and *BRCA* and are mainly considered to arise *de novo* from serous tubal intraepithelial carcinoma or ovarian serous inclusion cysts. These tumors have an aggressive clinical course. High-grade serous carcinomas are further divided into two subtypes: 1. classic (75%) and 2. solid, endometrioid-like and transitional (SET) type (25%). Classic type expresses *BRCA1/2* germline mutations in about 15% of cases. SET tumors show *BRCA1/2* germline mutations in up to 50%.

BRCA genes encode tumor suppressors taking part in homologous recombination. If mutated, alternative pathways of damaged DNA reparation are being activated, including one through base excision repair by poly ADP ribose polymerase (PARP), which can partly stabilize the genome. *BRCA* mutations are identified by molecular testing.

BRCA mutations increase sensitivity to platinum-based chemotherapy. By inhibiting PARP, the genome is left unstable and tumor cells are being destroyed. Nowadays, also in Croatia, PARP inhibitor is accessible for the treatment of high-grade serous carcinoma. Appropriate candidates for the PARP inhibitors treatment are women with recurrent disease and proven *BRCA* mutation, who have previously reached partial or complete response on platinum-based chemotherapy during at least 6 months. Clinical studies show that PARP inhibitor olaparib treatment improves progression free survival for 82% and prolongs median of overall survival for more than 3 months.

HEREDITARY LEIOMYOMATOSIS AND RENAL CELL CARCINOMA (HLRCC): CASE REPORT

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INTRODUCTION: Hereditary leiomyomatosis and renal cell carcinoma is a rare autosomal-dominant hereditary syndrome. Clinical manifestations are skin leiomyomas, multiple symptomatic uterine leiomyomas and early onset of type 2 papillary renal cell carcinomas which are usually aggressive and metastasize even when small. HLRCC is caused by heterozygous germline mutations of the fumarate hydratase (FH) gene on chromosome 1q34. Treatment of RCC should be prompt with wide-margin surgical excision and retroperitoneal lymph node dissection. At present, no standard therapy has been developed for patients with metastatic type 2 papillary RCC associated with germline FH mutations, and the overall survival for these patient is still limited.

CASE REPORT: A 48-year old woman presented with a left kidney mass. Two years earlier she had a hysterectomy because of uterine leiomyomas. Computed tomographic scan showed a 4,5 cm large solid mass of the left kidney. In October 2015 the patient underwent a left laparoscopic partial nephrectomy. Pathological studies revealed polymorphic renal cell carcinoma (high grade papillary NOS with mucinous tubular and spindle cell component), TNM staging was pT3aN2Mx. In November 2015 radical nephrectomy and lymphadenectomy was performed and confirmed metastases in regional lymph nodes. Additional molecular genetic study indentified pathogenic change on 3rd exon:c.301C>T, mutation in FH gene (Plzen, Czech Republic). An MRI of the spine confirmed a metastatic deposit at L3 and paliative radiotherapy was performed. Following surgical treatment the patient received temsirolimus 25mg/daily in 11 cycles and zometom. In October 2016 CT scan showed progression of disease in lungs, right adrenal gland, and bones. Between April and October 2016 the patient received sorafenib and nivolumab. Since November 2016 supportive therapy was applied and the patient died in December 2016.

CONCLUSION: We reported an interesting case of HLRCC, which is a rare syndrome associated with germline mutations in the gene for the Krebs cycle enzyme β FH. Estimated lifetime renal cancer risk for FH mutation carriers is 15%. Predictive genetic testing should be offered from ages 8-10. Mutation carriers should be offered a yearly MRI of kidneys in order to detect very small tumours.

THE COMPARATIVE PATHOLOGY STUDY OF FELINE AND CANINE MAMMARY TUMORS P53 AND P63 IMMUNOHISTOCHEMICAL EXPRESSION

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Mammary tumors are common canine and feline neoplastic pathologies. The prognosis varies significantly according to the degree of tumor invasion. Since myoepithelial layer destruction is considered a differential diagnosis parameter of in situ from invasive lesions in human breast cancer, we aimed to explore and evaluate the immunohistochemical expression of p63, a protein specifically expressed by myoepithelial cells of mammary gland. Furthermore its correlation with p53 expression was analyzed. The TP53 gene has the properties of a tumor suppressor gene and is the most frequently affected site of genetic alterations in human malignancies. It encodes the p53 protein in control of expression of a variety of genes involved in cell cycle regulation.

The 10 tumor specimens (8 canine and 2 feline tumors) were allotted three groups according to tumor grade: (G1) benign tumors; (G2) mammary carcinomas without metastasis at the diagnoses complex carcinomas; (G3) metastatic mammary carcinomas, solid carcinomas and complex carcinoma. All dogs and cats were females, ranging from 6 to 16 years of age. The number of p53 and p63 reactive cells was assessed semiquantitatively by two pathologists (S.Đ., A.D.) using a score system: 0 = no staining, 1 = between 1 and 50% of stained cells (low staining) and 2 = tumors with >50% stained cells (high staining) as has been proposed in literature. The statistical analysis by Fisher's Exact test was applied for the comparisons between different groups and p53 and p63 expression. The p63 nuclear staining was detected only in mammary myoepithelial cells of both species. The staining intensity gradually decreased from tumors without metastasis to those with metastasis, as lesion progressed. We also observed, as some other investigators, staining absence in malignant tumors with metastasis at the moment of diagnosis. There were no significant differences between groups by Fisher's Exact Test ($p < 0.05$). We have confirmed that p53 staining has always been demonstrated in high quantities in malignant tumors. However, we agree that p53 mutations should not be considered a unique event that initiates the process of carcinogenesis. The strong association between p53 and p63 was verified by Fischer's Exact Test, which indicated that the variables are statistically independent ($p > 0.05$), while the Spearman

Rank coefficient correlation between p53 and p63 showed that these two variables are statistically independents ($p < 0.05$).

To conclude, in the canine and feline groups of mammary tumors investigated we haven't observed species specific immunoexpression differences that would suggest a difference in molecular oncogenic pathways activation.

EFFICACY ASSESSMENT OF NEW CHEMICAL ENTITIES IN PATIENT DERIVED XENOGRAPTS ON EXAMPLE OF TGF β SIGNAL PATHWAY IN HUMAN COLORECTAL CANCER**M. Antolić¹, V. Eraković Haber¹, Ž. Javorščak¹, D. Marković¹, I. Glojnarić¹, L. Patrlj^{2,3}, R. Kliček², M. Kopljar², G. Aralica^{2,3}, A. Pačić², S. Čužić¹**¹Fidelta Ltd, Zagreb,²Clinical Hospital Dubrava, Zagreb,³Medical School University of Zagreb, Zagreb, Croatia

INTRODUCTION: TGF β signaling pathway has been shown to play an important role in the development and growth of colorectal carcinoma. Decreased TGF β R1 expression can potentially modify risk of colorectal cancer in humans (Calon et al, 2012). Relative to the surrounding intestinal mucosa, Smad7 expression in human colorectal cancer cells is significantly increased (Yan et al, 2011). Silencing of Smad7 inhibits the growth of colorectal cancer cell lines both in vitro and in vivo after transplantation into immune-deficient mice (Stolfi et al, 2014). Aim of this study was to test expression of proteins participating in TGF β signaling pathway, pSMAD3, MMP13 and TGF β 1 using immunohistochemistry and SMAD7 mRNA by in situ hybridization, in six samples of human colorectal cancer and patient derived xenografts (PDX).

MATERIAL AND METHOD: Human colorectal tumor samples were implanted subcutaneously in 5 week old BALB/c nu Immunodeficient Mouse (Janvier Labs). The development of xenografts was followed up to 18 weeks per generation, when xenograft tissue was transferred in next mice generation, Fo-F4. Expression of proteins was evaluated by immunohistochemistry, while Smad7 mRNA level was determined by in situ hybridization.

Tested primary antibody/antigen retrieval/staining kits were:

a) Immunohistochemistry

1. Target retrieval solution (10x) pH6, Cat.No. S1699, Lot 10112841; Exp.Date 05/2017
2. Anti pSMAD3, Cat.No. 039600-401-919, ROCKLAND, Lot 33229; Exp. Date 15.3.2018
3. Anti MMP13, Cat.No. NBP1-45723, Novus Biological; Lot 9A213533, Exp. Date 16.3.2018
4. TGF β 1, Cat.No. bs-0086R, Bioss, Lot 9l24M2; Exp. Date 16.3.2018

b) In situ hybridization

1. Target Retrieval Reagents, Cat.No.322000, RNA scope[®], Lot. 15300A, Exp. Date 31.3.2018.
2. Pretreat Reagents- H₂O₂ and Protease Plus Reagents, RNA scope[®], Cat.No.322330, Lot.15299A-R, Exp. Date 15.04.2017
3. Probe-Hs-SMAD7, Cat.No.419141, Lot.1525, Exp. Date 09.12.2017

Protein expression level was evaluated using score, a product of signal intensity score (0-3) and distribution score (1-4). Similarly, presence of Smad7 mRNA was expressed using score, total score was a product of intensity (0-4) and distribution score (1-4).

RESULTS: Expression of TGF β in primary colorectal tumors was low. From three primary colorectal carcinomas TGF β signal expression of in 3 tumors remained low through tumor generations, while in the other three tumors was variable. TGF β expression correlated with uttermost effector molecule of TGF β signal pathway, MMP13 as well as with pathway mediators' signal levels, Smad7 mRNA and pSmad3 protein. When signal expression of TGF β was low, SMAD7 mRNA was low, pSMAD3 intensity was high, as well as MMP13 protein level.

CONCLUSION: We have showed that in PDX model evaluation of molecules in TGF β signal pathway by IHC and ISH could be useful in testing of potentially effective new chemical entities targeting that signal pathway.

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METASTATIC SPREAD OF CANINE PERIANAL MELANOMA - A CASE REPORT**L. Medven Zagradišnik, A. Gudan Kurilj, I.-C. Šoštarić-Zuckermann,
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Melanocytic tumors are relatively frequent tumors of the skin and oral cavity in older dogs, and their biological behaviour often depends on their location. Oral tumors, including mucocutaneous tumors of oral cavity, are mostly malignant, contrary to skin tumors which are usually benign. Perianal melanoma, although mucocutaneous, has still unknown prognosis due to rarity of this tumor in dogs.

This report describes a case of a 13-year-old mixed breed bitch. The animal had a perianal nodular mass and excisional biopsy was preformed. The tumor was diagnosed as melanocytoma. Two years later, a locally recurrent mass was found with constipation as the only clinical sign. By fine-needle aspiration and cytological analysis of the mass diagnosis of suspected melanoma was made. The dog was euthanized and the necropsy showed a perianal exophytic black nodule. Also, a large mass in subsacral area of the pelvic cavity was revealed. Multiple tumor nodules were found at the serosal surfaces of stomach, gallbladder, rectum, urinary bladder and omentum. Likewise, lung metastases were found. The diagnosis of perianal melanoma with metastatic spread was made by histopathological examination. Immunohistochemical staining for Melan A and Ki-67 was performed, both on initial melanocytoma and subsequent melanoma, retrospectively, to determine the possibility of malignant transformation of this tumor.

To our knowledge this is a first necropsy report of metastatic spread of canine perianal melanoma. Thus, this case presents the malignant behaviour of a melanoma located at an unusual location for dogs, similar to such tumors in humans.

A CASE OF PLEOMORPHIC UNDIFFERENTIATED SOFT TISSUE SARCOMA IN PATIENT WITH LONGSTANDING INFLAMMATORY BOWEL DISEASE

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INTRODUCTION: The role of immunosuppressive therapy in the development of malignant disease has been controversial. From an immunological point of view it is plausible that inhibition of the immune system might hinder its tumor-suppressing functions.

Inflammatory bowel disease (IBD) has been associated with an increased risk of development of extraintestinal malignancies. This risk is largely attributed to IBD treatment modalities, namely thiopurines and anti tumor necrosis factor (TNF) agents, and includes lymphoproliferative disorders and skin cancers, including melanoma. Patients with IBS also might have an increased risk in developing thyroid and breast cancer.

CASE REPORT: We present a case of a 40-year-old woman, smoker, with longstanding IBD, diagnosed at age 20 with mild remitting gastrointestinal symptoms until age 35, responsive to therapy with topical and systemic 5-aminosalicylic acid. Concurrently, an autoimmune thyroiditis developed. The patient had a history of a fast growing inguinal mass over the course of a few months. On ultrasound examination, an hypoechoic formation measuring 30 mm in diameter was found. Cytology puncture showed poorly differentiated tumor cells resembling melanoma. An additional excisional biopsy was performed and a well circumscribed tumor was found, measuring 35 mm in diameter, fleshy on cut surface with foci of hemorrhage and necrosis. On microscopic examination, tumor consisted of anaplastic and pleomorphic cells with visible atypical mitoses. Neoplastic cells showed strong positivity for vimentin, CD 34, while muscle markers such as caldesmon and desmin were negative, as well as HMB 45, melan A, CK-PAN and S 100. In accordance with microscopic and immunohistochemical analysis a diagnosis of undifferentiated pleomorphic sarcoma was made. The margins of sample were positive and reexcision was performed, with foci of deep residual tumor.

Radiological visualisation methods did not show the presence of metastasis at the time of diagnosis. The patient is currently undergoing systemic oncological therapy.

CONCLUSION: Pleomorphic undifferentiated soft tissue sarcoma is an aggressive sarcoma of soft tissue or bone that can arise from any part of the body. Most undifferentiated high grade sarcomas occur in patients over age 40. We have presented a case of undifferentiated pleomorphic sarcoma in a patient with longstanding IBD. The possible association of mesenchymal malignancies and IBD is poorly understood, and more detailed studies are needed to shed light on this issue.

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INTRODUCTION OF FLUORESCENCE IN SITU HYBRIDISATION (FISH) ANALYSIS IN OUR ROUTINE MUCOEPIDERMOID CARCINOMA DIAGNOSTICS

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Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor (SGT), presenting 2-16% of all and 12-29% of malignant SGTs. It occurs most frequently in the parotid gland (45%) and within the palatal minor salivary glands. Ectopic salivary glands, as well as any other seromucinous gland, may be affected, including the bronchial glands. Median age of involvement is 45 years. MEC is the most frequent malignant salivary gland tumor occurring in children. This malignancy exhibits varying degrees of differentiation and histologic grade as well as widely diverse biologic behavior.

The t(11;19)(q21;p13) translocation involving the MECT1 and MAML2 genes has been identified as a diagnostic marker in mucoepidermoid carcinoma. There are two most common ways to detect chromosomal translocation t(11;19)(q21;p13): RT-PCR analysis of MECT1-MAML2 fusion gene expression and FISH analysis of MAML2 gene using break apart probe which enables detection of MAML2 rearrangement. We recently incorporated FISH analysis of MAML2 gene rearrangement in routine diagnostics of mucoepidermoid carcinoma. Having in consideration the rather small number of analysed samples so far, first results are encouraging. It is very important to have MAML2 gene rearrangement diagnostics available for mucoepidermoid carcinoma, not only for diagnostic purposes, but also as a prognostic factor, which some studies confirmed.

SYNCHRONOUS OCCURRENCE OF TWO PRIMARY NEOPLASMS WITH DIFFERENT HISTOTYPE IN THE SAME KIDNEY: A CASE REPORT**A. Mataić¹, M. Livojević², M. Ulamec^{3,5}, I. Tomašković⁴, B. Krušlin^{3,5}**

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BACKGROUND: The synchronous occurrence of two primary renal neoplasms is uncommon. Although hybrid oncocytic/chromophobe tumors (HOCT) of the kidney have been described in patients with Birt-Hogg-Dubé syndrome (BHD) and in association with renal oncocytosis without BHD, neither collision tumor nor two separate tumors with those histopathologic features have yet been described in the kidney.

CASE REPORT: A 74-year-old female was admitted to the urology department due to a kidney tumor. CT scan showed a hyperdense lesion in the lower pole of her left kidney measuring 6.7x5.7x8.2 cm, and another hypodense lesion in the median pole measuring 4.2x3.6x3.5cm. Nephrectomy was performed and three tumor masses were found. The largest tumor mass with a diameter of 8 cm was composed of cells with features consistent with chromophobe renal cell carcinoma. These cells were CD117, CK7 and von Hippel Lindau positive but AMACR and vimentin negative. Two smaller tumors with a diameter of 2,7 cm and 1 cm were composed of cells with features of renal oncocytoma. Oncocytes were CD117 and von Hippel Lindau positive but AMACR and CK7 negative. No pathogenic mutations were found in the *FCLN* genes.

CONCLUSION: There are different theories concerning the histogenetic basis of hybrid tumors and collision tumors. Hybrid oncocytic/chromophobe tumors are characterized by the association of both oncocytes and chromophobe cells within the same tumor, while collision tumors refer to the phenomenon where two apparently different and unrelated tumor types are present within the same location in an organ, forming a single discrete lesion. Our case is unique because the tumors were separate masses in the same kidney and we considered them as synchronous tumor.

References:

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3. Morelli, L, et al. Concurrent occurrence of three primary neoplasms with different histotype in the same kidney, associated with an adenoma of the omolateral adrenal gland: First case report. *Int J Urology*, 2006; 13: 1236-96.

**BASAL CELL CARCINOMA WITH STRONG SEBACEOUS DIFFERENTIATION -
CASE REPORT****D. Müller¹, D. Mužinić², T. Bota³, Ž. Tomasović-Lončarić¹**¹Department of Pathology, University Hospital Dubrava, Zagreb, Croatia²Department of Pathology, University Hospital Merkur, Zagreb, Croatia³Department of Pathology, General Hospital Virovitica, Virovitica, Croatia

The 82-year old female patient was admitted to our Hospital because of hip fracture. During her hospital stay a large tumor on her right auricle was noticed so she underwent surgical resection. The removed tumor has been histologically proven as basal cell carcinoma with strong sebaceous differentiation.

Basal cell carcinoma is by far the most common skin carcinoma. The architectural growth pattern is the only proven histologic prognostic factor of its clinical behavior. Many different histological patterns must be recognized as part of the broad spectrum of basal cell carcinoma, because they impact differential diagnosis. Basal cell carcinoma with sebaceous differentiation has to be distinguished from sebaceoma and especially sebaceous cell carcinoma. Sebaceous carcinoma with basaloid cells resembles basal cell carcinoma with sebaceous differentiation. Basaloid cells in basal cell carcinoma with sebaceous differentiation show typical peripheral palisading and retraction artifact from the adjacent stroma by clefts, which is not seen in sebaceous carcinoma. Basal cell carcinoma lacks the typical lobular architecture of sebaceous carcinoma. Several markers are differentially expressed in sebaceous carcinoma compared with basal cell carcinoma with sebaceous differentiation and the most useful panel of immunohistochemical stains consists of EMA, BerEP4 and Androgen. Sebaceous carcinoma is positive for EMA and androgen receptors, but negative for Ber-Ep4, while basal cell carcinoma is predominantly negative for EMA and androgen receptors, but positive for Ber-Ep4. These immunohistochemical stains were particularly useful in this case and helpful in identification of sebaceal differentiation of basal cell carcinoma.

Appendix

**KLINIČKI BOLNIČKI CENTAR OSIJEK
KLINIČKI ZAVOD ZA PATOLOGIJU I SUDSKU MEDICINU**

Predstojnik Kliničkog Zavoda
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Popis molekularnih analiza solidnih tumora - OSIJEK

Vrsta analize (gena)	Uzorak (materijal) na kojem se radi	Molekulama analize (uredaj)	EQA
Mutacije RAS i BRAF gena (adenokarcinom debelog crijeva) KRAS ekson 2 (kodoni 12, 13) ekson 3 (kodoni 59, 60, 61) ekson 4 (kodoni 117, 146) NRAS ekson 2 (kodoni 12, 13) ekson 3 (kodoni 59, 60, 61) ekson 4 (kodoni 117, 146) BRAF ekson 15 (kodon 600)	Tumorsko tkivo uklopljeno u parafin (mikrodisekcija)	Cobas® KRAS FFPET Mutation Test v2 i Cobas® BRAF/NRAS FFPET Mutation Test (Cobas z480)	Ne
Mutacije EGFR gena (adenokarcinom pluća) ekson 18 G719X (G719A, G719C, G719S) ekson 19 delecije, kompleksne mutacije (kombinacija delecije i insercije) ekson 20 S768I, T790M, insercije ekson 21 L858R, L861Q	Tumorsko tkivo uklopljeno u parafin (mikrodisekcija) Citološki razmaz	Cobas® EGFR Mutation Test v2 (Cobas z480)	Da, 2016 (ESP Lung EQA 2016 scheme for EGFR analysis)
Mutacija BRAF gena (melanom) ekson 15 (kodon 600)	Tumorsko tkivo uklopljeno u parafin (mikrodisekcija)	Cobas® 4800 BRAF V600 Mutation Test (Cobas z480)	Ne
Mutacije ALK gena (adenokarcinom pluća) mutacija D5F3	Tumorsko tkivo uklopljeno u parafin	Ventana BenchMark Ultra	Da, 2016 (ESP Lung EQA 2016 for ALK Testing in NSCLC)
In situ hibridizacija HER-2 (rak dojke i želuca)	Tumorsko tkivo uklopljeno u parafin	Inform HER2 Dual ISH (Ventana BenchMark Ultra)	Da, 2008 (Run B6 2008 NordiQC)

KLINIČKI BOLNIČKI CENTAR RIJEKA ZAVOD ZA PATOLOGIJU, LABORATORIJ ZA MOLEKULARNU DIJAGNOSTIKU

Krešimirova 42, 51000 RIJEKA

Popis molekularnih analiza solidnih tumora - RIJEKA

Vrsta analize (gena)	Uzorak (materijal) na kojem se radi	Molekularna analize (uređaj)	EQA
<p>Mutacije RAS gena (adenokarcinom debelog crijeva)</p> <p>KRAS ekson 2 (kodoni 12,13) ekson 3 (kodoni 59,60,61) ekson 4 (kodoni 117,146)</p> <p>NRAS ekson 2 (kodoni 12,13) ekson 3 (kodoni 59,60,61) ekson 4 (kodoni 117,146)</p> <p>BRAF ekson 15 (kodon 600)</p>	Tumorsko tkivo uklopljeno u parafin (radi se mikrodisekcija)	<ul style="list-style-type: none"> Sanger sekvencioniranje (ABI 310) Real time PCR LighMix Kit NRAS/KRAS, Molbiol (LightCycler, cobas z480) KRAS Mutation Test v2 (Cobas z480) 	Da, 2016 (The European Molecular Genetics Quality Network-EMQN)
<p>Mutacije EGFR gena (adenokarcinom pluća)</p> <p>ekson 18 G719X (G719A,G719C,G719S), ekson 19 delecije, kompleksne mutacije (kombinacija delecije i insercije) ekson 20 S768L, T790M, insercije ekson 21 L858R, L861Q</p>	Tumorsko tkivo uklopljeno u parafin (radi se mikrodisekcija) Citološka stakla (struže se samo dio sa tumorskim stanicama)	<ul style="list-style-type: none"> Cobas EGFRv2 (Cobas z480) RealLine EGFR Detect, Bioron (Applied biosystems 7500) Sanger sekvencioniranje (ABI 310) 	Da, 2015 (The European Molecular Genetics Quality Network-EMQN)
<p>Mutacija BRAF gena (melanom) ekson 15 (kodon 600)</p>	Tumorsko tkivo uklopljeno u parafin (radi se mikrodisekcija)	<ul style="list-style-type: none"> Sanger sekvencioniranje (ABI 310) BRAF Standard DNA kit, Bioron (Applied biosystems 7500) 	Da, 2015 (The European Molecular Genetics Quality Network-EMQN)
<p>Mutacije BRCA1 i BRCA2 gena (karcinoma ovarija) Somatske i zametne mutacije</p>	Tumorsko tkivo uklopljeno u parafin (radi se mikrodisekcija) Periferna krv	<ul style="list-style-type: none"> NGS (sekvencer nove generacije) 	U postupku vanjske kontrole, 2017 (The European Molecular Genetics Quality Network-EMQN)
<p>In situ hibridizacija HER-2 dojka n-MYC neuroblastoma</p>	Tumorsko tkivo uklopljeno u parafin (označena područja)	<ul style="list-style-type: none"> Vysis probe (FISH ili Ventana SISH) Dako probe (FISH) 	Da, 2016 (NordiqC)

**KLINIČKI BOLNIČKI CENTAR SPLIT
KLINIČKI ZAVOD ZA PATOLOGIJU, SUDSKU MEDICINU I CITOLOGIJU
LABORATORIJ ZA KLINIČKU GENETIKU**

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Popis molekularnih analiza solidnih tumora - SPLIT

Karcinom	Gen koji se analizira	Uzorak/materijal	Metoda/uređaj za analizu/testovi	Vanjsko testiranje kvalitete (EQA)
Kolorektalni karcinom	KRAS (28 mutacija) - ekson 2 (kodoni 12,13) - ekson 3 (kodoni 59,61) - ekson 4 (kodoni 117,146) NRAS (25 mutacija) - ekson 2 (kodoni 12,13,18) - ekson 3 (kodoni 59,61) - ekson 4 (kodoni 117,146) BRAF (11 mutacija) - ekson 11 (kodoni 466,469) - ekson 15 (kodoni 600,601)	Tumorsko tkivo uklopljeno u parafin	Real time PCR / Cobas z 480 (Roche) - KRAS Mutation Test v2 (LSR) - BRAF/NRAS Mutation Test (LSR)	ESP EQA 2010. IRIS EQA 2017. - u tijeku
Adenokarcinom pluća	EGFR (42 mutacije) - ekson 18 (kodon 719) - ekson 19 (delecije i kompleksne mutacije) - ekson 20 (kodoni 768,790) - ekson 21 (kodoni 858,861)	Tumorsko tkivo uklopljeno u parafin Citološki razmazi Periferna krv (ctDNA)	Real time PCR / Cobas z 480 (Roche) - Cobas EGFR Mutation Test v2	ESP EQA 2014. ÖGPath/ IAP 2015.
Melanom	BRAF (11 mutacija) - ekson 11 (kodoni 466,469) - ekson 15 (kodoni 600,601)	Tumorsko tkivo uklopljeno u parafin	Real time PCR / Cobas z 480 (Roche) - BRAF/NRAS Mutation Test (LSR)	Ne
Serozni karcinom jajnika visokog gradusa	BRCA1 i BRCA2	Tumorsko tkivo uklopljeno u parafin Periferna krv	Masivno paralelno sekvenciranje - do 31.12.2017. 454 GS Junior (Roche) - od lipnja 2017. MiniSeq (Illumina)	Da, 2016 (ESP Lung EQA 2016 for ALK Testing in NSCLC)
Karcinom dojke	HER-2	Tumorsko tkivo uklopljeno u parafin	SISH/BenchMark Ultra (Ventana)	NordiqC 2013.

**LABORATORIJ ZA MOLEKULARNU PATOLOGIJU
ZAVODA ZA PATOLOGIJU MEDICINSKOG FAKULTETA I
KLINIČKOG ZAVODA ZA PATOLOGIJU I CITOLOGIJU KBC-a ZAGREB**

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Popis molekularnih analiza solidnih tumora - ZAGREB

Tumori	Geni/promjene	Uzorak/materijal	Metoda/uređaj za analizu/testovi	Vanjsko testiranje kvalitete (EQA)
Adenokarcinom debelog crijeva	KRAS - 28 mutacija: ekson 2 (kodoni 12 i 13) ekson 3 (kodoni 59 i 61) ekson 4 (kodini 117 i 146) NRAS - 25 mutacija: ekson 2 (kodoni 12, 13 i 18) ekson 3 (kodoni 59 i 61) ekson 4 (kodoni 117 i 146) BRAF - 11 mutacija: ekson 11 (kodoni 466 i 469) ekson 15 (kodoni 600 i 601)	Tumorsko tkivo uklopljeno u parafin	Real time PCR Cobas z 480 - KRAS Mutation Test v2 (LSR) - BRAF/NRAS Mutation Test (LSR)	ESP EQA 2010. ESP EQA 2012. ESP EQA 2017. u tijeku IRIS EQA 2017. u tijeku
Adenokarcinom pluća (dr. nesitnostanični karcinom na zahtjev)	EGFR - 42 mutacije: ekson 18 (kodon 719) ekson 19 (delecije i kompleksne mutacije) ekson 20 (kodoni 768 i 790; insercije) ekson 21 (kodoni 858 i 861)	Tumorsko tkivo uklopljeno u parafin Citološki razmaz Periferna krv (ctDNA)	Real time PCR Cobas z 480 - Cobas EGFR Mutation Test v2	ÖGPath/ IAP 2012. ESP EQA 2017. u tijeku
Serozni karcinom jajnika visokog gradusa	BRCA1 i BRCA2 - svi eksoni; prijelazi ekson-intron	Tumorsko tkivo uklopljeno u parafin Periferna krv	Masivno paralelno sekvenciranje Ion Torrent PGM - Ion AmpliSeq BRCA1 and BRCA2 Panel	
Karcinom grlića materice	Detekcija HPV-a uz genotipizaciju: - niskog stupnja rizika (6 i 11) - visokog stupnja rizika (16 i 18)	Tumorsko tkivo uklopljeno u parafin Bris cerviksa	PCR - "In house"	

Karcinom usne šupljine	Detekcija HPV-a uz genotipizaciju: - niskog stupnja rizika (6 i 11) - visokog stupnja rizika (16 i 18)	-Tumorsko tkivo uklopljeno u parafin	PCR - "In house"	
Papilom grkljana	Detekcija HPV-a uz genotipizaciju: - niskog stupnja rizika (6 i 11)	Tumorsko tkivo uklopljeno u parafin	PCR - "In house"	
Mukoepidermoidni karcinom	Preraspodjela MAML2	Tumorsko tkivo uklopljeno u parafin	FISH - ZytoLight SPEC MAML2 Dual Color Break Apart Probe	
NUT karcinom središnje ravnine	Translokacija BRD3-NUTM1 Translokacija BRD4-NUTM1	Tumorsko tkivo uklopljeno u parafin	RT-PCR - "In house"	
Melanom	BRAF - 11 mutacija: ekson 11 (kodoni 466 i 469) ekson 15 (kodoni 600 i 601)	Tumorsko tkivo uklopljeno u parafin	Real time PCR Cobas z 480 - BRAF/NRAS Mutation Test (LSR)	
Ewingov sarkom	*Translokacije EWSR1-FL11 *Translokacija EWSR1-ERG **Preraspodjela EWSR1	Tumorsko tkivo uklopljeno u parafin (*/**) Periferna krv (*) Koštana srž (*)	*RT-PCR - "In house" **FISH - Vysis EWSR1 Break Apart FISH Probe Kit (CE)	
Osteosarkom	Amplifikacija MDM2	Tumorsko tkivo uklopljeno u parafin	FISH - Vysis MDM2/CEP 12 FISH Probe Kit (CE)	
Aneurizmatička koštana cista	Preraspodjela USP6	Tumorsko tkivo uklopljeno u parafin	FISH - ZytoLight SPEC USP6 Dual Color Break Apart Probe	
Sinovijalni sarkom	Translokacija SS18-SSX1 Translokacija SS18-SSX2	Tumorsko tkivo uklopljeno u parafin	RT-PCR - "In house"	
Alveolarni rabdomiosarkom	Translokacija PAX3-FOXO1 Translokacija PAX7-FOXO1	Tumorsko tkivo uklopljeno u parafin	RT-PCR - "In house"	

Miksoidni liposarkom	*Translokacije FUS-DDIT3 **Preraspodjela FUS	Tumorsko tkivo uklopljeno u parafin (*/**)	*RT-PCR - "In house" **FISH - Vysis FUS Break Apart FISH Probe Kit (CE)	
Dobro diferencirani liposarkom	Amplifikacija MDM2	Tumorsko tkivo uklopljeno u parafin	FISH - Vysis MDM2/CEP 12 FISH Probe Kit (CE)	
Dezmoplastični tumor malih okruglih stanica	*Translokacija EWSR1-WT1 **Preraspodjela EWSR1	Tumorsko tkivo uklopljeno u parafin (*/**)	*RT-PCR - "In house" **FISH - Vysis EWSR1 Break Apart FISH Probe Kit (CE)	
Fibromiksoidni sarkom niskog stupnja malignosti	*Translokacija FUS-CREB3L1 *Translokacije FUS-CREB3L2 **Preraspodjela FUS	Tumorsko tkivo uklopljeno u parafin (*/**)	*RT-PCR - "In house" **FISH - Vysis FUS Break Apart FISH Probe Kit (CE)	
Sarkom svijetlih stanica	*Translokacije EWSR1-ATF1 *Translokacije EWSR1-CREB1 **Preraspodjela EWSR1	Tumorsko tkivo uklopljeno u parafin (*/**)	*RT-PCR - "In house" **FISH - Vysis EWSR1 Break Apart FISH Probe Kit (CE)	
Neuroblastom	Amplifikacija MYCN Delecija u kromosomu 1 (1p)	Tumorsko tkivo uklopljeno u parafin	FISH - Vysis LSI N-MYC (2p24) Spectrum-Green/Vysis CEP 2 SpectrumOrange Probe (ASR) - Vysis LSI 1p36 / LSI 1q25 and LSI 19q13/19p13 Dual-Color Probe (CE)	
Oligodendrogliom SŽS-a	Delecija u kromosomu 1 (1p) i u kromosomu 19 (19q)	Tumorsko tkivo uklopljeno u parafin	FISH - Vysis LSI 1p36 / LSI 1q25 and LSI 19q13/19p13 Dual-Color Probe (CE)	
Meduloblastom	Amplifikacija MYCC Amplifikacija MYCN	Tumorsko tkivo uklopljeno u parafin	FISH - Vysis LSI N-MYC (2p24) Spectrum-Green/Vysis CEP 2 SpectrumOrange Probe (ASR) - Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probe Kit (CE)	



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