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The abstracts are the author's responsibility.

OP1. DYSPLASIA IN INFLAMMATORY BOWEL DISEASE – SURVEILLANCE AND ENDOSCOPIC TREATMENT

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Introduction: It is well known that the risk for colorectal cancer (CRC) is increased in patients with Inflammatory Bowel Disease (IBD) when compared with the general population, and it depends essentially of the patients' age, family history, activity and extent of the disease. Dysplasia is the most reliable marker of malignancy in IBD. Nowadays, endoscopic procedures play a prominent role in the treatment of such patients.
Methods: A total of 591 lower endoscopies executed in the surveillance program of CRC in IBD patients were included (from 2010 to 2014). Sporadic dysplastic lesions were excluded.
Results: A total of 25 adenomatous lesions with dysplasia were excised in 20 patients (11 men, 9 women), 16 with Ulcerative Colitis (Proctitis – 1, Left Colitis – 9, Pancolitis – 6) and 4 with Crohn's Colitis. Their average age was 59.9 ± 14.2, with about 14.3 years of disease duration. When the procedure was undertaken, all patients were taking 5-ASA and 4 of them were under immunosuppressive drugs (20%). This group was significantly older than the one with no adenomas (59.9 *vs* 48.6; p = 0.001). The two groups were similar in gender and duration of disease. All lesions had low grade dysplasia, except for one that was a serrated adenoma, and were treated by endoscopy: polypectomy in 15 patients, endoscopic mucosal resection in 10. Average size of the lesion was 14.4 mm (4-40 mm). A complete resection was achieved in 18 procedures (R0 – 72%). There were no severe complications. The patients were followed for an average of 28.8 months, with at least one follow-up exam. One of the lesions relapsed, but it was successfully retreated as well. There was no need for surgery and no cases of CRC in this group of patients.
Conclusion: This group of patients with non sporadic adenomas was significantly older than those without adenomas. Some years ago, a significant part of these patients had to undergo colectomy. Currently, endoscopic procedures play a part not only in the surveillance of these high risk patients, but also in treating these lesions, as it has few complications and low relapse rate.

OP2. BARRETT'S ESOPHAGUS: CURRENT CLINICAL PICTURE

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Introduction: Barrett's esophagus (BE) is a known precursor to esophageal adenocarcinoma. The aim of this study was to evaluate the characteristics of BE in our clinical practice, given the paucity of information on this topic in the scientific literature.
Methods: Retrospective study including all patients with the diagnosis of BE in biopsies during upper endoscopy (UE) performed in a hospital unit for January 2005-January 2014. Review of epidemiological, clinical (gastroesophageal reflux symptoms: heartburn/acid regurgitation), endoscopic (short/long BE segment: < 3 cm/3 cm), histologic (using Vienna classification), therapeutic and monitoring data following American Gastroenterological Association recommendations.
Results: Of the 21,992 UE performed, 92 were in 42 patients with histological diagnosis of BE (histological detection BE rate: 0.4%). This population had a mean age – 60.9 years; male sex -71.4%; hiatus hernia-57.1%. At the time of UE, 77.8% was under anti-reflux therapy. History of gastroesophageal reflux symptoms present in

73.6%. Short BE found in 53.6%. Histologically, metaplasia types: intestinal-87.5%, cardia-10%, fundic-2.5%. Only 33.3% underwent endoscopic follow-up according to the standard recommendations. In the first endoscopic evaluation, we identified the following histological findings, all in long BE: 1 low grade dysplasia; 5 invasive carcinomas (IC) - 2 at an early stage. During follow-up, were identified: 1 high grade dysplasia (DAG); 2 IC - 1 at an early stage. The patient with DAG and 2 patients with early IC underwent endoscopic resection, both required to repeat the procedure. The remaining IC underwent surgery. There were two deaths: one by metastatic disease despite surgical therapy; and another by a cardiovascular event. Mean follow-up: 85 months.
Conclusion: Despite the long follow-up and that BE has been identified histologically in few patients, the number of high-grade dysplasia/carcinoma cases was significant. However, the surveillance recommendations were not fulfilled in most cases.

OP3. DIFFERENT RESPONSE PROFILES OF RADIOSENSITIVE AND RADIORESISTANT COLORECTAL CANCER CELL LINES

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Introduction: Chemoradiation as neoadjuvant treatment for locally advanced rectal cancer is one of the most common approaches, since it allows downstaging and improves local control. To understand how radioresistant cells behave after treatment is extremely important in order to improve treatment modalities. In this study we aimed to compare cell viability, DNA damage, oxidative stress and GSH expression of sensitive parental colorectal cancer cells to its radioresistant derivatives, after treatment with 5-fluorouracil (5-FU), radiation alone and combined therapy.
Methods: Parental WiDr cell line and the radioresistant derivative WiDr/10r, previously obtained by our group, were used to study radioresistance. Cells were submitted to 20 μM of 5-FU for chemotherapy and irradiated with 0, 2 and 10 Gy for radiation alone, in a Varian Clinac 600 linear accelerator with a 4MV photon beam. Cells treated with combined therapy were exposed to 20 μM of 5-FU three hours prior irradiation and then irradiated with the same doses. Cell viability and oxidative stress were assessed by flow cytometry 96 hours after treatment and DNA damage was measured by comet assay immediately after treatment.
Results: WiDr cells showed a significant decrease of cell viability after exposure to 10 Gy and to 5-FU + 10 Gy, comparing to their derivative radioresistant cells WiDr/10x (respectively 49.1 ± 5.0% vs 66.0 ± 8.1%, p = 0.008; and 50.9 ± 2.4% vs 63.8 ± 4.2%, p = 0.003) and a significant increase of necrosis, regarding 10 Gy (30.9 ± 8.5% vs 19.3 ± 4.2%, p = 0.001), 5-FU (22.5 ± 5.2% vs 17.8 ± 2.7%, p = 0.009) and 5-FU + 10 Gy (27.0 ± 6.6% vs 16.2 ± 2.3%, p = 0.015). Although there were no differences in superoxide anion concentration, WiDr cell line showed a significant decrease in formation of peroxides when compared to WiDr/10x after treatment with 5-FU + 10 Gy (160.0 ± 10.0% and 200.0 ± 10.0% respectively, p = 0.006). WiDr cell line exhibited lower mitochondrial membrane potential than WiDr/10x cell line, i.e., superior monomers/aggregates ratio, after treatment with 10 Gy and 5-FU (540.0 ± 80.0% vs 360.0 ± 50.0%, p = 0.012; and 230.0 ± 30.0% vs 180.0 ± 30.0%, p = 0.015, respectively). DNA damage was superior in WiDr/10x cell line than in WiDr cell line, since the tail moment parameter was significantly higher in the first one for all treatment conditions (p < 0.001).
Conclusion: We were able to establish radioresistant cell lines from WiDr cell line. These results showed that radioresistant WiDr/10r cell line is less affected by higher doses of radiation, regarding cell viability and oxidative stress. However, radioresistant cell line acquired more DNA damage than radiosensitive cell line, which means that cells can carry DNA damage and still be viable and resistant to treatment.

OP4. RESECTION OF HEPATIC AND LUNG COLORECTAL METASTASES: EXPERIENCE OF A SINGLE CENTER

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Introduction: Multidisciplinary approach (MA) of patients with colorectal cancer (CRC) has allowed to increase, very significantly, the survival of patients with hepatic (HM) and lung (LM) metastases. We evaluated the results of liver and lung metastases of colorectal cancer (MCRC) resection in our specialized surgical unit between 2006-2014.
Methods: Twenty-nine patients underwent liver and lung resection by MCCR [17 men, mean age 62 ± 8.8 years (47-81)]. The most common location of the primary tumor was the rectum (55%), and the LM were synchronous with the primary tumor in eight patients (metachronous in 21); LM were synchronous with HM in 38% (n = 11) and metachronous in 62% (n = 18). LM's were unilateral in 83% and 55.2% were single. All patients underwent neoadjuvant or adjuvant chemotherapy.
Results: Operative mortality (3 months) was zero; 13 patients were re-operated for pulmonary recurrence. Survival was 100% and 89% at 1 year and 73% and 38% at 5 years after treatment of primary tumour and LM respectively. Actuarial survival was higher in patients with unilateral LM (p = 0.023). No statistical difference was seen in survival of patients treated with synchronous or metachronous LM.
Conclusion: In selected patients, resection of LM and HM of CRC can significantly prolong survival and an aggressive MA strategy should be adopted.

OP5. IMMUNE RESPONSE, RADIATIOTHERAPY AND LUNG CANCER

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Lung cancer (LC) is responsible for a considerable number of deaths. The aim of this study was to assess the effects of X radiation (X-rays) in three lung cancer (LC) cell lines after irradiation, two of non-small cell lung cancer (H1299 and A549 cells) and one of small cell lung cancer (H69 cells). Viability, proliferation, type of cell death and cell cycle were studied. Additionally we also studied some of the mechanisms involved, namely P53 expression, the role of oxidative stress and mitochondrial membrane potential (MMP). For clinical studies, blood samples from 17 patients with LC were collected in three distinct stages: before (T0), half (T1) and 30 days after radiotherapy (RT) (T2) to characterize the different lymphocyte populations $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD4^-CD8^-$, $CD3^+CD4^+CD8^+$, $CD19^+$, $CD3^+CD56^+$, $CD3^+CD56^+CD8^+$ and $CD4^+CD25^+CD127^+FOXP3^+$, and the levels of the following molecules GRO-α, ING-γ, IL-1RA, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12p70, IL-13, IL-18, IL-27, IP-10, MCP-1, MIP -1α, MIP-1β, RANTES, SDF-1,TNF-α were evaluated. In *in vitro* studies we observed an inhibition of cell proliferation for all three cell lines, in a time and dose-dependent manner, being apoptosis the main type of cell death which was associated with a decrease in MMP. Regarding the clinical study, it was observed an increase of $CD4^+$ cells at the time T0 to the time T1 and a decrease at the time T2, in conjunction with $CD4^+CD8^+$ cells which make the cross-talk between cells of the innate and acquired immune-system. An explanation for this fact is their recruitment to the tumor site. Our results suggest that the removal of the tumor may influence the presence of lymphocyte subsets in peripheral blood. Taking together the results we think that this work has contributed to clarify the role of the immune-system, molecules produced by him and by the microenvironment.

OP6. CR(VI)-INDUCED SENESCENT BRONCHIAL STROMA STIMULATES EPITHELIAL TO MESENCHYMAL TRANSITION ON BRONCHIAL EPITHELIAL CELLS EXPOSED TO CR(VI)

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Introduction: Cellular senescence is a physiological process that serves as a powerful barrier for tumorigenesis. However, senescent cells can be deleterious for the tissue microenvironment. Such is the case of senescent fibroblasts that release several pro-tumorigenic factors promoting malignant transformation in the nearby epithelial cells. Occupational exposure to hexavalent chromium [Cr(VI)] compounds is a cause of respiratory cancers. Although Cr(VI) is known to induce senescence in human foreskin fibroblasts, the role of senescent fibroblasts in the Cr(VI)-induced malignant transformation of human bronchial epithelial cells was never assessed.
Methods: Primary cell cultures of human bronchial fibroblasts (HBFs) were established from lung biopsies obtained following surgical resection. Then, HBF cultures were chronically exposed to several Cr(VI) concentrations (0.25 to 2.0 μM) during different periods of time to determine the ideal exposure regimen to induce senescence on these cells. Aiming to understand the effects produced by interaction between the senescent HBFs and human bronchial epithelial cells, the nontumorigenic human bronchial epithelial BEAS-2B cells were co-cultured with Cr(VI)-induced senescent HBFs for 4 weeks. Alterations on cell morphology and expression of significant biomarkers were analyzed in BEAS-2B cells and HBFs by immunocytochemistry.
Results: A fibroblast primary cell culture (designated E2A) was successfully obtained from lung biopsies. The exposure of E2A to 0.5 μM Cr(VI) for 4 weeks induced morphological changes, e.g. loss of elongated form and acquisition of a round and flattened shape. Cells' growth arrest and the increase of β-galactosidase activity showed by Cr(VI)-exposed E2A confirmed the acquisition of a senescent phenotype by these cells. In parallel a myofibroblastic cell subpopulation expressing α-SMA emerged within E2A cultures exposed to Cr(VI). As regards to co-cultured cells, under the pressure of 0.5 μM Cr(VI), senescent fibroblasts induced on BEAS-2B cells the acquisition of mesenchymal features, such as the fusiform shape and increased Vimentin expression, consistent with the occurrence of an epithelial to mesenchymal transition-like process. Features of transformed cells including larger nuclei, as well as nuclei with heterogeneous size, were also observed.
Conclusion: In sum, the results obtained revealed that Cr(VI) acts not only over the epithelium, but also induces a senescent phenotype on bronchial fibroblasts. Consequently, a paracrine communication loop is established with the above-placed epithelium prompting the epithelial cells for malignant transformation and thus facilitating the initial steps of tumorigenesis. Having in mind that respiratory airways are lined with epithelial cells, the primary target for inhaled particles toxicity including Cr(VI), we propose two models to explain how Cr(VI) may reach the fibroblasts. Thus, based on our results, in sites of high Cr(VI) concentration, epithelial cells' death is induced enabling the compound to diffuse through the basement membrane and attain stromal fibroblasts that become senescent. Instead, in sites of lower Cr(VI) concentration epithelial cell-cell adhesions are degraded allowing Cr(VI) to diffuse through intercellular clefts, reaching fibroblasts and inducing their senescence. The senescence-associated secretory phenotype acquired by fibroblasts will stimulate the malignant transformation of epithelial cells that survive to the Cr(VI) insult. The inflammatory environment induced by Cr(VI) may promote the recruitment of macrophages to these sites that will play a crucial role on basement membrane degradation favoring Cr(VI) diffusion towards stromal fibroblasts.

OP7. RESISTANCE EXERCISE AT 70% OF MAXIMUM RESISTANCE INCREASES THE CIRCULATING NUMBER OF ENDOTHELIAL PROGENITOR CELLS: PRELIMINARY RESULTS

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Introduction: Strenuous or maximal aerobic exercise acutely stimulates the mobilization of endothelial progenitor cells (EPCs) from the bone marrow, which may be sustained up to 2-3 days. However, it is unclear whether other exercise modalities such as resistance training could change the circulating number of EPCs both in health and disease. Hence, this study aimed to investigate the acute effects of a single bout of resistance exercise performed on the circulating number of EPCs.
Methods: Twelve university students (mean age: 21 ± 0.9 years old; height: 1.63 ± 0.07 meters; weight: 56.2 ± 10.5 kg; BMI: 21.1 ± 2.4 kg/m^2) volunteered to participate in this study. Subjects visited the lab twice, once for assessment of muscle strength to determine maximal resistance for each muscle group that was going to be exercised in the exercise session, and once (at least 48h after the first visit) for the exercise session. The resistance exercise session

was performed at 70% of 1 maximum repetition and comprised 3 sets of 12 repetitions of the following exercises: bench press, dumbbell curl, dumbbell squat, and standing dumbbell upright row. The subjects rested 1 minute between sets and the session lasted approximately 30 minutes. Venous blood was collected at baseline, immediately after exercise, and 6 and 24 hours postexercise. To evaluate EPCs in the peripheral blood by flow cytometry, whole blood samples were labelled with monoclonal antibodies against CD34, CD309, and CD45. Identification of the EPCs was based on morphological properties and CD45-CD309+CD34+ profile.
Results: There was a statistically significant difference in circulating EPCs depending on the time of measurement ($F_{3,33}$ = 33.273, $p < 0.001$; $\eta^2_p = 0.752$). The post-hoc analysis indicated a 42.7 ± 19.2% increase in the number of EPCs from baseline (0.007652 ± 0.001452%) to immediately after exercise (0.010946 ± 0.002790%, $p < 0.001$) and a 23.0 ± 13.5% increase at 6h postexercise (0.009384 ± 0.001867%, $p < 0.001$); and a small (11.2 ± 12.0%), but significant decrease in EPCs at 24h postexercise (0.006792 ± 0.001535%, $p < 0.001$) compared to baseline.
Conclusion: A single session of resistance exercise at 70% of maximum resistance induced an acute increase in the circulating EPCs that was sustained up to 6 hours after the exercise termination.

OP8. DECODING GENETIC AND EPIGENETIC SIGNATURES IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction: Oral squamous cell carcinoma (OSCC) is characterized by low survival and high mortality rates, mostly due to the frequent diagnosis of these tumors in advanced stages and the development of loco-regional recurrences. In this sense, despite significant progress in cancer treatment, early detection of these tumors and its curable precursors remains the best way to ensure patient survival and improved quality of life. Taking into account that genetic and epigenetic alterations may occur before phenotypic ones, molecular profiling holds great promise to help in the early diagnosis and also to predict disease progression.
Methods: Biopsies of oral tumors were acquired from 73 OSSC patients and gingival samples from 11 healthy donors were used as controls. Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) was conducted to screen copy number alterations and DNA methylation patterns in 54 tumor suppressor genes using two probe panels.
Results: We found that in our tumor cohort, from the 54 tumor suppressor genes included in this study, those that most frequently exhibited hypermethylation were *WT1* (54/73), *MSH6* (24/73), *PAX5* (24/73) and *GATA5* (17/73). The most frequent copy number alterations were located at chromosomes 3, 9, 11, 12, 16, 17 and 19. In these chromosomes several imbalances were highlighted namely, losses at *CTNNB1* (27/73) and *FHIT* (25/73) genes and gains at *CDH1* (33/73), *BRCA1* (25/73), *PYCARD* (24/73), *STK11* (23/73) and *CHFR* (20/73) genes.
Conclusion: Based in the rational that cancer is the result of the accumulation of genetic and epigenetic aberrations, the analysis of these imbalances is essential in order to establish biomarkers capable to identify tumors in early stages. The combination of genetic and epigenetic studies together with the pathological diagnosis seems to be mandatory not only to early detect these tumors and relapses but also to predict their behavior. In this way, the increasing knowledge about the molecular mechanisms associated to oral cancer, opens new possibilities at the diagnostic, prognostic and treatment level.

OP9. RELEVANCE OF MICRORNAS EXPRESSION IN RESPONSE TO TARGETED THERAPIES IN CHROMIC MYELOID LEUKEMIA

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Introduction: Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by the translocation t(9:22), resulting in *BCR-ABL* fusion gene encoding the BCR-ABL oncoprotein with increased tyrosine kinase activity. Despite the success of treatment with Imatinib, it has been observed an increase in patients with incomplete responses or resistance to this drug. The BCR-ABL oncoprotein is able to activate multiple signaling pathways, including the NF-kB, mTOR and the proteasome pathways, which may provide therapeutic alternatives in cases of resistance to Imatinib. Furthermore, the altered expression of microRNAs, small RNA molecules that regulate gene expression, can influence the sensitivity and/or acquisition of resistance to therapy, particularly to Imatinib. In this work, we evaluated the influence of the expression levels of miR-21, miR-125b and miR-155 in CML cell lines sensitive and resistant to Imatinib, as well as the therapeutic potential of new drugs such as Bortezomib, Parthenolide and Everolimus.
Methods: To achieve our goals we used two CML cell lines, K562 cells (Imatinib-sensitive) and K562 RC cells (Imatinib-resistant). Cell viability was assessed by rezasurine assay and cell death by flow cytometry (annexin V and propidium iodide) and by optic microscopy (May-Grünwald-Giemsa staining). The expression of BAX, BCL-2, phosphorylated NF-kB, ubiquitin conjugates, p53 and cell cycle analysis was performed by flow cytometry. The expression levels of AKT and activation were analyzed by Western blot and expression of miRNAs was performed by qRT-PCR using commercial kits.
Results: Initially it was found that K562 RC cells show increased expression of miR-21 and miR-125b and decreased expression of miR-155 in relation to K562 cells. Bortezomib, Parthenolide and Everolimus induced a decrease in cell viability in a time-, dose- and sensitivity to Imatinib-dependent manner. It was observed that Parthenolide and Bortezomib induce a stronger cytotoxic effect on K562 RC cells, while Everolimus shows a higher cytotoxic effect on K562 cells sensitive to Imatinib. These results may be related to the differential expression of miRNAs in the cell lines. These compounds induce cell death predominantly by apoptosis. In addition, Everolimus induced the cell cycle arrest in G2/M phase. On the other hand, treatment with these compounds is capable of modulating the expression of miRNAs, which can influence cell drug response.
Conclusion: In summary, Bortezomib, Parthenolide and Everolimus may be new therapeutic approaches in CML. Moreover, miR-21, miR-

125b and miR-155 expression levels could provide new biomarkers predictive of response to TKI and/or new targeted drugs such as Bortezomib, Parthenolide and Everolimus in CML.
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OP10. ENDOMETRIAL CANCER STEM CELLS: P53 EXPRESSION AND RESPONSE TO RADIOTHERAPY

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Introduction: Endometrial cancer, the most common gynecologic malignancy in western countries, is usually symptomatic and diagnosed in initial stages. However, the risk of recurrence is largely influenced by prognostic factors, particularly molecular characterization. Cancer stem cells (CSC), a small proportion of tumor cells, have self-renewal capacity and generate a differentiated progenitor cells that origin the majority of tumor cells. The characterization of this population in endometrial cancer can contribute to stratify clinical prognosis and early diagnosis
Methods: Endometrial cancer cell line ECC1 was submitted to sphere forming protocol. The first spheres generation (ES1) was cultured in adherent conditions. This procedure was repeated in order to obtain successive generations of spheres (ES1, ES2 and ES3) and the spheres-derived cells in adherent conditions (G1, G2 and G3). The expression of P53 was evaluated on each generation obtained. These cell populations were submitted to radiation (0.5, 15 and 30 Gy) and metabolic activity was evaluated using MTT and Alamar-Blue assay at 24, 48 and 72 hours.
Results: There was a significant decrease of P53 expression in the spheres population (ES1, $p = 0.006$; ES2, $p = 0.006$; ES3, $p < 0.001$) comparing with endometrial cell line, with no significant differences among the 3 generations. In adherent populations derived from these cells there are no differences in P53 expression comparing to parental cell line of origin, emphasizing a similar phenotype. These results point a deficient expression of tumor suppressor proteins in endometrial stem cell population. In general, irradiation had a low effect, inferior to 10%, on the metabolic activity of the endometrial cell line ECC-1. However, after 72h there is a decrease to 74.3 ± 3.9% with 15Gy and 76.3 ± 4.1% with 30 Gy. Regarding sphere derived adherent populations (G1, G2 and G3), similar results were found comparing with ECC-1 cell line. The first sphere population ES1 irradiated with 30Gy at 72h had a metabolic activity of 86.9% ± 4.8%. The following generations of cancer stem cells (ES2 and ES3) showed an equivalent response to irradiation. This points that spheres populations are more resistant to irradiation than adherent-derived populations that appear to harbor a phenotype regression.
Conclusion: The expression of P53 is decreased in stem cells population comparing with endometrial cell line and adherent derived population. The irradiation of endometrial cancer has modest impact on proliferation. A group of cells with stem cell properties seems to resist to irradiation.