10. Cancer Pharmacology

10.001 Melatonin receptors as pharmacological targets for glioma therapy. Kinker GS[1], Oba-Shinjo SM[2], Carvalho-Sousa CE[1], Muxel SM[1], Marie SKN[2], Markus RP[1], Fernandes PA[1]
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Introduction: Gliomas, the most common primary brain tumors in adults, exhibit poor responses to standard treatment and are associated with high mortality. We have recently demonstrated that the ability of such tumors to synthesize/accumulate melatonin negatively correlates with their overall malignancy (Kinker et al., J Pineal Res, 60, 84, 2016). Using luzindole, a non-selective antagonist of melatonin membrane receptors, we have shown that glioma-synthesized melatonin exerts an autocrine anti-proliferative effect. Additionally, based on The Cancer Genome Atlas (TCGA) glioma RNAseq data, we have designed a two-gene predictive model of the content of melatonin in the tumor microenvironment, combining the gene expression levels of melatonin synthesis and metabolism enzymes. The ASMT:CYP1B1 index negatively correlates with tumor grade and represents an independent prognostic factor: a low score, suggestive of reduced melatonin, is strongly associated with poor survival. Therefore, to further characterize the pathophysiologic relevance of the melatonergic system of gliomas, here we investigated the specific roles of melatonin receptors MT1 and MT2 in the oncostatic actions of this indolamine. **Methods**: We evaluated the expression of melatonin membrane receptors, MT1 and MT2, in human glioma cell lines with different grades of aggressiveness (HOG < T98G < U87MG), by imaging flow cytometry. Cell lines were treated with DH97 (10^{-10} – 10^{-6} M, 48 h), an MT2-selective antagonist (pKi = 8.03, 89-fold selectivity over MT1), and cell number was estimated by MTT assays. Using the TCGA RNAseq and clinical data of 624 patients, we evaluated the association between the overall malignancy of gliomas and the expression of MT1 and MT2 receptors. **Results**: All cell lines expressed MT1 and MT2 and, surprisingly, the selective blockage of MT2 receptors by DH97 10^{-8} M significantly reduced the growth of HOG and T98G, less aggressive cell lines which synthesize/accumulate more melatonin. Interestingly, this oncostatic effect was reverted, in a concentration dependent manner (DH-97 10^{-7} – 10^{-6} M), by the concomitant inhibition of both melatonin receptors. In fact, the treatment with DH97 10^{-6} M mimicked the effects of the non-selective antagonist luzindole and stimulated the growth of HOG and T98G (15% greater than control), suggesting that MT1 and MT2 have opposite roles in tumor progression. Accordingly, the TCGA RNAseq data analysis revealed that the expression of MT2 alone is more frequent in high-grade (30%) than in low-grade (15%) gliomas. More importantly, among patients with a high ASMT:CYP1B1 index score, those expressing only MT1 presented a decreased expression of cell cycle progression genes and survived 1.7 times longer than those expressing only MT2. Conclusion: Overall, our data reinforce the prognostic value of the melatonergic system of gliomas, supporting further investigations of the biological relevance of tumors-synthesized melatonin. Additionally, we lay a substantial groundwork for the use of melatonin analogous that activate MT1 and/or inhibit MT2 receptors in glioma therapy. **Financial support**: FAPESP (2010/52687-1, 2013/13691-1, 2014/27287-0), CNPq (480097/2013-5, 162670/2014-1).

10.002 Bioprospection of compounds isolated from *Combretum fruticosum* **with antiproliferative potential in tumor cells.** Moura AF[1], Lima KSB[2], Sousa TS[2], Marinho-Filho JDB[1,3], Pessoa CO[4], Silveira ER[2], Pessoa ODL[2], Moraes MD[1], Araújo AJ[1,3] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Organica, [3]UFPI – Curso de Medicina, [4]Fiocruz

Introduction: The use of substances from natural products has grown over the years, being the basis of therapeutic products. Lignans are molecules with large pharmaceutical use, which has aroused interest in search of new drugs to treat diseases. Much interest has been focused on their effectiveness as an antineoplastic agent (LEE; XIAO, 2003). Thus, the aim of this study was to evaluate the *in vitro* anticancer potencial of compounds isolated from ethanolic extract of *Combretum fruticosum*, as well as, to study the possible mechanisms of action of a dibenzylbutyrolactone type lignan, Trachelogenin (**TRA**), in colorectal cancer cells. **Methods:** Compounds were obtained from ethanolic extract of *C. Fruticosum* stalks, collected in Caucaia County. After isolation, purification and identification of the bioactive compounds, the antiproliferative activity of them was evaluated by MTT assay. To study the possible mechanism of action involved in antitumor activity of **TRA** in HCT-116 cells, it was performed cell viability assessing using Trypan blue exclusion assay, analyzing cell morphology by optical and confocal microscopy, studying cellular events by flow cytometry and protein expression by *Western blot*. **Results:** The fractionation of the ethanolic extract of *C. fruticosum* resulted in the isolation of seven compounds: three triterpenes, two mixtures of β-sitosterol and stigmasterol steroids and two lignans. Among them, the lignan, Trachelogenin (**TRA**) showed higher cytotoxic activity, with IC_{50} values ranging from 0.8 to 32.4 µM in glioblastoma (SF-295) and leukemic (HL-60) cells, respectively. While in normal cells (3T3-L1 and PBMC cells) the IC_{50} values were greater than 64.3 µM. The antiproliferative profile of different times of incubation was performed in SF-295 and HCT-116 cells. The cytotoxic effect on SF-295 cells was only observed after 72 hours of incubation, whereas in HCT-116 cells, this effect was observed after 48 hours, and it was enhanced after 72 hours of incubation. Before these results, analyzing cell cycle profile, membrane integrity, phosphatidylserine externalization and expression of proteins related to cell death by apoptosis in HCT-116 cells, it was not observed significantly changed, suggesting that the antiproliferative effect of this lignan is not related to mechanisms of cell death such as apoptosis and/or necrosis. Autophagy seems to be one of the cell death mechanisms involved in the antiproliferative effect of **TRA**, because we observed an increase on number and size of acidic vesicular organelles (AVO) as well as the expression of proteins recruited during autophagy (LC3 A and B-II and Beclin-1) in cells treated with **TRA**, although this seems not to be the only process involved. **Conclusion:** Therefore, Trachelogenin showed potent antitumor activity *in vitro*, and this effect may be related to the induction of autophagy. However, further tests should be conducted to confirm these proposals and to evaluate its mechanism of action and the therapeutic potential of this molecule better. **Supported by:** CNPq, PRONEX, CAPES and FUNCAP. **References:** LEE, H. K.; XIAO, **Phyto. Rev.**, v. 2, p. 341, 2003.

10.003 Antitumoral activity of ethanolic extract and the diterpene Fruticulin A of *Salvia Lachnostachys* **in Ehrlich Solid Carcinoma model in mice.** Corso CR[1], Stipp MC[1], Adami ER[1], Oliveira CS[2], Stefanello MEA[2], Acco A[1] [1]UFPR- Farmacologia, [2]UFPR- Química

Introduction: Among the agents studied for new approaches to the cancer treatment, the natural compounds have been extensively investigated. *Salvia L.* genus is gaining therapeutic value due to the various reported biological properties, among them the antitumoral activity. Thus, this study aimed to investigate the antitumoral effects of ethanolic extract (EES) and the diterpene fruticulin A (Fruti), from *Salvia lachnostachys,* in Ehrlich solid carcinoma in mice. **Methods:** Ehrlich cells were maintained in ascitic form by intraperitoneal passages ($2x10^6$ cells/mice) in female mice (~25-30 g) until cells reach ≥98% viability (by Tripan blue method). To evaluate a possible reduction on cell viability, animals were intraperitoneally treated with vehicle (distilled water), EES 30 mg/kg, EES 100 mg/kg, Fruti 3 mg/kg and Methotrexate (MTX) 2.5 mg/kg, during 5 days and tumor cells were counted on day 6. For solid model, Ehrlich cells were inoculated subcutaneous in the right pelvic member ($2x10^6$ cells/mice). Animals were orally treated, as reported above, from 21 days after tumor inoculation. Tumor volume was measured on day 7 until day 21. On day 22, animals were euthanized, liver and tumor were collected and parameters of oxidative stress (GSH, glutathione reductase; GST, gluthatione S-transferase; SOD, superoxide dismutase; CAT, catalase and LPO, lipid peroxidation) were analyzed; tumor anti-inflammatory parameters (MPO, myeloperoxidase; NAG, N-acetylglucosaminidase and NO, nitric oxide); antiangiogenic activity (tumor hemoglobin levels); and morphological analysis were also performed. Data were analyzed using one-way ANOVA followed by Bonferroni pos hoc test (n= 6-10). **Results:** EES30 did not reduce tumor volume, instead EES100 and Fruti showed reduction from day 10 (63% and 61%, respectively) until day 21 (41% and 51%, respectively), when compared to vehicle group. MTX showed a pronounced reduction on tumor volume from day 7 (99%) until day 21 (90%). However, only Fruti diminished tumor weight (45%). Furthermore, only EES100 reduced tumor SOD activity (44%). No effect was observed on cell viability with EES or Fruti treatment, indicating no direct antitumoral effect on tumor cells. EES (30 and 100) and Fruti showed a reduction of tumor MPO activity (29%, 38% and 20%, respectively) and NO levels (47%, 31% and 41%, respectively), while no reduction was observed in NAG activity. Moreover, a reduction on tumor hemoglobin was observed only with Fruti treatment (49%). Histological analysis showed increase in necrosis cells on Fruti treatment compared to vehicle group. Morphometric quantifications are further analyzed as well as genic expression of proteins involves in necrosis, inflammation and angiogenesis. **Conclusion:** These results showed possible anti-inflammatory and antiangiogenic activities of Fruticulin, resulting in antitumoral activity by inducing tumor cells necrosis. More studies are in process to better elucidate the fruticulin mechanism of action. **Acknowledgements:** C.R.C. is recipient of a doctorate scholarship from CAPES. **Ethical Committee:** All experimental procedure were approved under the nº 879 (UFPR).

10.004 Effect of TrkB Selective Blockade in A172 glioblastoma cells. Pinheiro KV[1], Silva CA[1], Gil MS[1], Duque MB[1], Thomaz ACG[1], de Farias CB[2], Roesler R[1] [1]UFRGS, [2]ICI-RS

Introduction: Glioblastoma Multiforme (GBM) is the most frequent primary malignant brain tumor diagnosed in adults. It is an aggressive neoplasm, associated with malignant clinical progression, characterized by widespread invasion of the whole brain tissue and high resistance to conventional therapies approaches. In this context it is essential the development of new therapeutic strategies to selective molecular targets for the treatment of cancer. **Methods:** The A172 cell line was obtained from the American Type Culture Collection (Rockville,Md., USA) were cultured in Dulbecco's modified Eagle'smedium (DMEM) low glucose supplemented with 10 % (v/v) fetal bovine serum (FBS) and 1%(v/v) penicillin/streptomycin. Cells were incubated in a humidified atmosphere of 5 % CO2 at 37 °C. The cells were seeded at a density of $5x10^3$ cells/well in 96-well plates and allowed to grow for 24h. The medium was replaced and cells were treated with increasing concentrations of ANA-12 (0, 1, 10, 20, 30 and 50 µM) for 48h. Cell viability was assessed by trypan blue cell counting and cell survival was measured with a colony formation assay. **Results:** Treatment with ANA-12 significantly reduced the viability of A172 cells assessed by trypan blue assay compared to control. Similarly, this treatment was able to promote a decrease in colony formation in cells. In both assays, the TrkB inhibitor ANA-12 effects was dose-dependently. **Conclusion:** These data support the view that inhibition of TrkB is a promising therapeutic target for the development of novel targeted therapies for the treatment of GBM. **Financial Support**: FIPE-HCPA, ICI-RS, CNPq, CAPES. **References** Cohen-Cory S, **Developmental Neurobiology** 70: 271-288, 2010. Desmet CJ, **Cell Mol Life Sci** 63:755–759, 2006. de Farias CB,**Biochem Biophys Res Commun** 425:328–332, 2012. Furnari FB. **Genes Dev**. 721: 2683-2710, 2007. Krüttgen A, Int. Soc. **Neurophatol Brain Phatol** 16:304-310, 2006. Taylor TE. **Curr Cancer Drug Targets** 12: 197–209, 2012. Thiele CJ. **Clin Cancer Res** 15:5962-5967, 2009. Thomaz ACG, **J Mol Neurosci**, 2015.

10.005 Mechanisms underlying the anti-tumor effects of quinoxaline-derived chalcones in oral squamous cell carcinoma. Mielcke TR[1], Erig TC[1], Chiela EC[2], Mascarello A[3], Chiaradia L[3], Nunes RJ[3], Campos MM[1] [1]PUCRS, [2]UFRGS, [3]UFSC

Introduction: Oral cancer is a worldwide public health problem. Treatment options are very limited, showing marked side effects. The tumor relapse is quite common, and therefore, new treatment options are required (Lubek et al., J Oral Maxillofac Surg. 71:1126, 2013). Previous evidence showed patent antiproliferative effects for eight quinoxaline-derived chalcones in glioma cells (Mielcke et al., Eur J Med Chem. 48: 255, 2012). This study examined the *in vitro* effects and the mechanisms of action of a series of 20 quinoxalinic chalcones in oral squamous cell carcinoma (OSCC). **Methods:** Human HN-30 and rat SCC-158 OSCC cells were seeded in 96-well plates and exposed for 24, 48 and 72 h to 20 different quinoxaline-based chalcones (0.5, 0.1, 1, 5 and 10 µg/ml). Cell viability was evaluated by MTT assay. The compounds named N9, N17 and N23 were selected for further experiments, based on their antiproliferative potential. HN-30 cells were exposed to N9, N17 and N23 (2.5 µg/ml), alone or in combination with subthreshold concentrations of the chemotherapy compounds 5-fluorouracil (1 µg/ml), cisplatin (3 µg/ml) or docetaxel (0.012 µg/ml). The cells were periodically counted for up to 26 days, and the cumulative population doubling (CPD) was calculated to assess the long-term effects of treatments. Flow cytometry was performed to determine the profile of cell death (annexin V-FITC-propidium-iodide and acridine orange staining), the effects on cell cycle (BrdU flow kit), or the modulation of Akt, Erk and p38 pathways (Phosflow Protocol for Adherent Cells). **Results:** From the 20 compounds, seven presented maximal inhibitions around 50% (at 5 µg/ml; during 48 h) in both tested cell lines. The three most effective compounds (N9, N17 and N23) were selected to perform mechanistic experiments. The CPD assay demonstrated that any isolated treatment failed to inhibit the cell proliferation during the follow-up period (throughout 26 days). Long-lasting antiproliferative effects were observed with the combination of N9 and N17, wherein the cell growth was observed only at 18 days. When the chalcones were associated with 5-fluorouracil or cisplatin, the growth was evident after 19 days of treatment. In the cell cycle analysis, N9 and N17 induced a significant increase of the cell population at the sub-G1/G1 phases, whereas the incubation of N23 increased the cell population at the G2 phase. The cell death induced by the three chalcones occurred via apoptosis/autophagy mechanisms, with prominent effects for N9. Preliminary data revealed an absence of significant effects for any tested chalcone on the activation of Akt, Erk and p38 pathways. **Conclusions:** The tested quinoxaline-based chalcones displayed either acute or long-term *in vitro* antiproliferative effects on OSCC, driving cell death via apoptosis/autophagy. The combination of chalcones with reference chemotherapy drugs showed favorable effects in long-lasting CPD protocols. Further experiments are in progress to assess the *in vivo* efficacy and the safety profile of these compounds. **Financial Support:** FINEP/PUCRSINFRA #01.11.0014-00; CNPq, CAPES, PUCRS

10.006 *In vitro* **Antiproliferative Effect Of 2-Quinoxalinyl-Hydrazones Derivatives In Tumor Cells** Maranhão SS[1], Moura AF[1], Sousa FCE[2], Luciano MCS[2], Paier CRK[2], Nepomuceno FWAB[3], Souza MVN[4], Pessoa CO[4] [1]UFC – Fisiologia e Farmacologia, [2]UFC, [3]UNILAB, [4]Fiocruz

Introduction: Quinoxalines are mostly synthetic low-weight heterocyclic compounds. These substances exhibit multiple biological activities that explain the growing interest of the pharmaceutical industry[1]. Among these activities may be mentioned the antineoplastic, anti-inflammatory, antibacterial, antiviral, antifungal, antiparasitic and anti-diabetic[2]. This study evaluated the cytotoxic effect of three synthetic quinoxalines on the viability of tumor cells and healthy cells. **Methods:** The determination of IC_{50} (inhibitory concentration of 50% of cell growth) was performed by the MTT and Alamar Blue colorimetric methods in function of time (24h, 48h and 72h). The compound with the best inhibitory activity was selected for further investigations, like proliferation and viability assay by Trypan blue; analysis by flow cytometry of membrane integrity, externalization of phosphatidylserine and DNA content; real-time monitoring of cell growth at XCelligence system. In order to verify the pattern of death induced by the molecule were performed confocal microscopy and analysis of activation of apoptotic proteins by Western blot. **Results:** All three tested Quinoxalines exhibited decrescent IC50s in function of time. Among them, PJOV 56 stands out for its antiproliferative activity against colorectal cancer cell line HCT-116 (IC50 at 24h, 48h and 72h: 40.02 ±2.09; 3.04 ±0.08 and 1.83 ± 0.03 µmol.L-1, respectively). This compound was selected for treatment at three concentrations (1.5, 3 and 6 µmol.L-1) for 48 hours in all the investigations. At the highest concentration (6 µmol.L-1), the analysis of phosphatidylserine externalization and caspase activation showed a significant percentage of apoptotic cells. Treatment with PJOV 56 showed changes in cell cycle distribution at 3 and 6 µmol.L-1, with accumulated cells at G1 and S phases, respectively. Cells treated under these concentrations presented an increase in cell size, vacuole formation, decrease in the number and increase in number and size of acidic vesicular organelles, verified by Panotic staining and confocal microscopy. **Conclusion:** The synthetic quinoxalines tested showed a cytotoxic effect against seven cancer cell lines. Among them, PJOV 56 showed a cytostatic nature at low concentrations, suggesting a senescent phenotype and in high concentrations induced apoptosis in cell line HCT-116. [1]AJANI, O. O. Euro. J. of Med. Chem., v.85, p.688, 2014. [2]PEREIRA, J. A. et al. Euro. J. of Med. Chem., v.97, p.664, 2014. **Financial agencies and acknowledgements:** CNPq, Funcap, UFC, CAPES.

10.007 Estrogen receptor ESR2 and beta-catenin mediate cell migration in androgen-independent prostate cancer cell PC-3. Lombardi APG, Vicente CM, Porto CS[1] [1]Unifesp – Endocrinologia

Introduction: Prostate cancer initially responds well to androgen-deprivation therapies, but the majority of tumors evolve from an androgen-sensitive to an androgen-independent form of the disease, also known as castration-resistant prostate cancer. The molecular mechanisms involved are still not well understood (reviewed by Parray et al., Biologics: Targets and Therapy 6:267, 2012). Recent studies from our laboratory have shown the expression of estrogen receptors ESR1 (ERalpha) and ESR2 (ERbeta) in PC-3 cells, used in vitro and in xenograft implants as CRPC models (Pisolato et al., Steroids 107:74, 2016). Furthermore, estrogen plays a role in PC-3 cell proliferation through a novel pathway, involving ESR2-mediated activation of beta-catenin (Lombardi et al., Mol Cell Endocrinol 430:12, 2016). It is important to emphasize that non-phosphorylated beta-catenin associates with transcription factor TCF/LEF-1 in the nucleus, and, together with co-activators, activate transcription of genes involved with cell proliferation and differentiation and epithelial-mesenchymal transition, which is thought to regulate the invasive behavior of tumor cells (reviewed by Lombardi et al., 2013). Thus, this study was performed to investigate whether ESR2 could play a role in PC-3 cell migration. **Methods:** PC-3 cells were grown in culture medium as previously described (Lombardi et al., 2016). Confluent cell monolayer was treated with a blocking DNA replication, mitomycin C (10µg/L, 30 min), and wound healing assay was performed. Briefly, a scratch was made using a 200 µl pipette tip and cells were washed with phosphate buffer saline to remove detached cells and debris. Photographs of the same area of the wound were taken at 0 and 24 h for measuring the closure of the wound after the treatment of the cells. Cells were incubated in the absence (control) and presence of E2 (0.1nM), ESR2-selective agonists DPN (10nM) and ERB-041 (10nM), ESR2-selective agonist PPT (10nM) for 24 hours. The cells were also untreated or pretreated with ESR2-selective antagonist PHTPP (10nM) and specific disruptor of β-catenin/TCF (PKF-118-310, 10nM), for 30 minutes. Afterwards, the cells are stimulated with agonists of ERs. Images were captured using a Nikon Eclipse inverted optical microscope and analyzed by Micrometrics SE Premium 4 software. **Results and Conclusions:** The treatment with E2, DPN and ERB-041 for 24 h caused an enhancement of 1.5-fold, 2.5-fold and 1.0-fold, respectively, in the cell migration. The treatment with PPT did not have any effect. As expected the pretreatment with PHTPP blunted the effect induced by DPN and ERB-041 in PC-3 cell migration, indicating the involvement of ESR2. The pretreatment with PKF 118-310 inhibited the cell migration induced by DPN, suggesting that beta-catenin is involved in this process. In the absence of DPN, the antagonists or the disruptor of beta-catenin did not have any effect on cell migration. These results suggest that estrogen may play a role in PC-3 cell migration through a novel pathway, involving ESR2-mediated activation of β-catenin. This study, together with our previous study, potentially identifies ESR2 and/or beta-catenin as possible new targets for prostate cancer treatment. **Financial Support**: FAPESP and CNPq

10.008 Effect of simvastatin on the MUC1 expression in vivo study of experimental mammary carcinogenesis Cardelli AJN[1], Belato KK[2], Coutinho SP[1], Rennó A[3], Franchi JG[4], Nowill A[5], Nascimento FC[6], Latuffi FP[4], Vassalo J[4], Malagoli RR[6], Souza BV, Schenka AA[2]
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Introduction: Statins inhibit HMG-CoA reductase, is used to reduces overall cholesterol levels, but they became important in pharmacology studies due to pleiotropic activities. Some studies demonstrated that statins can be associated to reducing of signals driving cell proliferation and survival responses. Thus, recent studies related that some statins (simvastatin) have a putative inhibitory effect on cancer stem cell (CSCs). **Objective:** Herein, we investigated if a well-know tumor antigen, MUC1 (recently tested as an immunotherapy target on cancer stem cells) is also expressed after *in vivo* simvastatin treatment. **Methods:** MCF7 cell line in xenografts was further subjected to immunophenotyping for $MUC1^+$ marker (a tumor antigen). Twenty NOD/SCID female received $3x10^6$ MCF-7 cells in one point of pad mammary. When the tumor volume was $1cm^3$, mice were randomly distributed into four groups of 6 animals each (24-48 hours). In each period of time, xenografts were dosed a single acute via oral gavage with 40mg/Kg simvastatin dissolved in soybean oil or administered soybean alone (vehicle control). At 24 and 48 hours after the treatment, mice were anesthetized and sacrificed. It was assessed histology of the samples and immunoexpression of MUC1. Experimental protocols were 2414-1. **Results:** In this study, it was observed that tumors showed diffuse growth, infiltrative and undifferentiated aspect (rare glandular formations). In addition, characteristics of malignancy were observed as extensive invasion in adipose and muscle tissue, severe cytological atypia, moderate nuclear pleomorphism, presence of nucleoli and high mitotic index (10HPF). Immunohistochemistry was used to assess the expression of MUC1 marker before and after simvastatin treatment. We observed that control group had high proportion of MUC1 (89%) and after 24 (80, 22%), 48hs, (80, 79%). MCF-7 tumor did consistently express MUC1 on their surface in all groups, with $P>0.05$. **Final Considerations:** We suggest that MUC1, a transmembrane glycoprotein, expressed (not at all) in high levels on the majority of carcinomas, maintain a $MUC1^+$ population with the stem/progenitor characteristics after simvastatin treatment. Financial Support: FAPESP, CAPES, FAEPEX References: **Gauthaman** K, Manasi N, Bongso A. Br J Pharmacol. **2009** Jul;157(6):962-73. doi: 10.1111/j.1476-5381.**2009**.00241.x. Epub **2009** May 11. Dimitroulakos J, Ye LY, Benzaquen M, Moore MJ, Kamel-Reid S, Freedman MH et al. (2001). Differential sensitivity of various pediatric cancers and squamous cell carcinomas to lovastatin-induced apoptosis: therapeutic implications. Clin Cancer Res 7: 158–167. Edwards PA, Ericsson J (1999). Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. Ann Rev Biochem 68: 157–185. Ezeh UI, Turek PJ, Reijo RA, Clark AT (2005).

10.009 Detection of the Breast Cancer Stem and Progenitor Cell Markers CD10 and CD133 after treatment with Simvastatin in MCF-7 xenografts. Belato KK[1], Cardelli AJN[2], Rennó A[3], Nascimento FC[4], Latuffi FP[5], Vassalo J[4], Malagoli RR[4], Souza BV, Schenka AA[1]
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Introduction: Cancer stem cells (CSCs) became a major endpoint in cancer research, since cumulative evidences suggest they could be responsible for tumor formation, morphological heterogeneity and treatment resistance in breast cancer.(Gauthaman, et al.,2009) Understanding the properties and characteristics of CSCs could lead to improvements in cancer diagnosis and therapeutical response. Simvastatin is an anti-dyslipidemic drug used to reduce overall cholesterol levels and whose main mechanism is represented by the inhibition of HMG-CoA reductase, limiting step in cholesterol biosynthetic pathway.(Gauthaman et al, 2009). In addition to its lipid lowering effects, recent studies have suggested simvastatin may have an inhibitory effect on cancer stem cell (CSCs). **Objective:** Herein, we aimed to investigated if the CD133 and CD10 (putative CSC antigens) expression in breast cancer xenografts could be modulated by in vivo treatment with simvastatin. **Methods:** Twenty-four NOD/SCID female mice received $3x10^6$ MCF-7 cells in one point of the pad mammary. When the tumor volume was $1cm^3$, the mice were randomly distributed into four groups of 6 animals each. Experimental groups 1 and 2 received a single oral dose of simvastatin (40mg/Kg) and were euthanized 24 or 48h, respectively. Control groups 1 and 2 received soybean oil (vehicle) instead, and were sacrificed at similar time points. MCF-7 xenografts were assessed for morphological features (H&E stained sections) and mean percentage of CD133 and CD10 positive cells (as detected by immunohistochemistry). All experimental protocols were approved by the local Ethics Committee (#2414-1). **Results:** We observed that tumors showed diffuse growth, atypical cells with nuclear pleomorphism, evident nucleoli and abundant cytoplasm and high mitotic index (10/HPF). Concerning the immunoexpression of CSC antigens, we observed that control animals had low counts of CD10 (37±2, 7) and that those were not significantly different (P>0,05) from experimental animals, regardless of time-point (16,7±1 at 24h and 23,29±2 at 48h). Similarly, CD133 had low count in control (25±7) and experimental groups (36,5±5 at 24h and 29,4±5 at 48h), with no statistically significant differences among between groups. **Conclusion:** We suggest that CD10 and CD133 expression in MCF-7 xenografts is consistently low, and cannot be acutely modified by a single high dose of simvastatin. Apoio Finandeiro: Fapesp, Capes e Faepex Referências: Kalamegam Gauthaman, Chui-Yee Fong, and Ariff Bongso (Statins, Stem Cells, and Cancer.Journal of Cellular Biochemistry 106:975–983; 2009. K Gauthaman, N Manasi and A Bongso (Statins inhibit the growth of variant human embryonic stem cells and cancer cells in vitro but not normal human embryonic stem cells British Journal of Pharmacology, 157, 962–973 2009.

10.010 Proteolytic fraction from *Vasconcellea cundinamarcensis* **latex induces differentiation in mouse melanoma B16F10 cell line.** Santos VG[1], Lemos FO[1], Salas CE[2], Lopes MTP[1] [1]ICB-UFMG – Farmacologia, [2]ICB-UFMG – Bioquímica e Imunologia

Introduction: *Vasconcellea cundinamarcensis* is a species of papaya tree, native plant of South America, whose latex is rich in cysteine proteases. Proteolytic fraction P1G10 obtained by chromatographic separation of latex, shows antitumoral/antimetastatic activity on different murine models. CMS2, a P1G10 sub-fraction, exhibits similar effects and increased protein expression related with melanoma cell differentiation, determinated by proteomic analysis. This study presents preliminary results on the CMS2 effects during the B16F10 melanoma cell differentiation *in vitro*. Here, we evaluate parameters related to cell transformation as melanogenesis induction, metalloprotease activity and ability to form colonies. **Methods and Results:** B16F10 cells were pretreated with CMS2 (1-10 µg/ml) for 2 - 8 days and seeded on 6 well plates ($4x10^5$ cells/well) to determinate melanin content or tyrosinase activity. To evaluate melanin production, cells were lysed with 1 M NaOH solution and were placed in a water bath at 80˚C for 2 hs. Detection was performed by measurement of the absorbance at 405 nm, which was then corrected for protein concentration. The tyrosinase activity was determined by L-DOPA oxidation method. Cells were lysed with 1% Triton X-100/PBS, centrifuged by 10,000 rpm, 10 min, 4°C, and to the supernatant was added L-DOPA (2mg/ml) and incubated for 2 hs at 37°C. The absorbance at 490 nm was measured and corrected by protein concentration. Stimulation of melanogenesis was time-dependent for both evaluated parameters. The melanin content in the cells treated with CMS2 (5 and 10 µg/ml) for 6 days, increased in 346% (446.0 ± 45.7 $p<0.0001$) and 191% (290.0 ± 4.3 $p<0.001$), respectively, compared with the melanin content of the control group (100.0 ± 18.1). The cells treated with CMS2 (5 or 10 µg/ml) exhibited increased tyrosinase activity about 21% ($p<0.05$), relative to the untreated group (100.0 ± 1.8). Besides, the gelatinase activity of metalloproteases (MMPs) was determined in B16F10 culture supernatant, by zymography method. The densitometric analysis of gels (software ImageJ® 1.46) showed that 5 or 10 µg/ml CMS2 treatments reduced the MMPs activity until 99% (0.7 ± 0.4 $p<0.0001$) relative to the untreated group (100.0 ± 6.5). The colony formation assay was performed with 200 cells/well, on 6-well plates, and after 13 days, colonies were washed with PBS and stained with 0.5% (w/v) crystal violet/ethanol 70% for 1 h and were counted (colonies containing more than 50 individual cells). CMS2 (5 and 10 µg/ml) showed a reduction of colonies number at 58% (12.5 ± 2.1 $p<0.001$) and 84% (4.8 ± 1.3 $p<0.0001$), respectively, relative to the untreated B16F0 cells (30.0 ± 6.1). Differences between groups were performed using the one-way ANOVA test followed by Student-Newman-Keuls *post-test*. **Conclusion:** These results indicated that CMS2 can promote the differentiation of B16F10 cells by melanogenesis induction, which consequently can affect the cell tumorigenicity, once were observed the reduction of colonies formation and metalloproteases activity. **Financial Support**: CNPq, CAPES and FAPEMIG.

10.011 Protective Effect of Ethanolic Extract of *Chuguiraga spinosa* **on DMBA-induced Breast Cancer in Rats** Arroyo JL[1], Herrera O[2], Chavez R[3], Anampa A[4], Chumpitaz V[5], Ruiz E[5], Rojas C[6] – [1]Universidad Nacional Mayor de San Marcos – Lima, Peru – Institute of Clinical Research / Pharmacology Laboratory, [2]Universidad Nacional San Luis de Gonzaga, Ica, Peru – Pharmacy and Biochemistry, [3]ADIECS-Universidad Nacional Mayor de San Marcos, Lima – Peru – Association for the Development of Student Research in Health Sciences, [4]Universidad Nacional Mayor de San Marcos, Lima – Peru –Medicine, [5]Universidad Nacional Mayor de San Marcos, Lima – Peru – Faculty of Odontology, [6]Universidad Nacional Mayor de San Marcos, Lima – Peru –Pharmacy and Biochemistry

Introduction: was the second leading cause of death in 2014 in the world. Chuquiraga spinosa Less (Huamanpinta) contains flavonoids and phenolic acids with strong antioxidant effect in vivo. This study aims to determine the protective effect of ethanolic extract of Chuguiraga Spinosa on DMBA-induced breast cancer in rats. **Methods**: Experimental study. 36 female Holtzman rats were housed in the bioterium of the Faculty of Medicine, National University of San Marcos. The rats were divided into six groups. Groups were named according to the treatment and dose in mg/kg. CS refers to the ethanolic extract of Chuquiraga spinosa. The control group received physiological saline (200 ml/kg body weight) orally. Group CS200 received CS (200 mg/kg body weight) as drug control. Group DMBA were induced with DMBA. The groups DMBA + CS50, DMBA + CS200, and DMBA + CS400 received CS (50, 200, and 400 mg/kg body weight, respectively) orally. Breast cancer in rats was assessed by monitoring the tumor and lung metastases incidence and recording hematological and biochemical parameters and frequency of micronuclei.Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey multiple comparison method was carried out to compare the mean value of different groups. A P-value of 0.05 was considered statistically significant in all cases. **Results**: Chuquiraga spinosa has 24 GAE mgr/gr of phenolic compounds. Oral administration of CS signifcantly lowered adenocarcinoma mass ($p < 0.05$). Also, CS significantly lowered CRP and SOD ($p < 0.05$) and showed dose-dependent effect. Hematological indicators showed that the glycemia, trygliceride and hepatic enzyme levels were significantly lowered ($p < 0.05$, $p < 0.05$ and $p < 0.05$ respectively). Toxic effects were not found. **Conclusion**: Considering the antitumorigenic, hypolipidemic, hipoglicemic, antioxidant, and antigenotoxic properties of the ethanolic extract of Chuquiraga spinosa Less, we conclude that it has a protective effect on DMBA-induced breast cancer in rats. **Financial support**: Authors disclose no funding sources. Research approval: The protocol was approved by the Institute for Ethics in Health of the National University of San Marcos (140103021)

10.012 Copaiba oil effects on evolution of Walker 256 tumor inoculated in the female rats bladder. Botelho NM, Leite GMO, Praia WC, Nunes MP, Dórea MA UEPA – Ciências Médicas

Introduction: Bladder cancer ranks ninth in the incidence of cancer in the world. It is more common in men and the worst initial prognostic factors are among the smokers. This cancer remains a challenging clinical problem because has a possibility to progress to a fatal disease. Therefore, alternatives forms of treatment for cancer are searched highlighting the use of medicinal plants such as Amazon plants. Thus, one of the best ways to test these new substances is use them against experimental tumors, such as carcinoma induction model of Walker 256. The aim of this study is evaluate the effects of copaiba oil (Copaifera reticulata) in the evolution of Walker 256 carcinoma inoculated in the female rats' bladder. **Methods**: The animals were randomized distributed into three groups: 1) Normal Group (NG) with animals not subjected to the injection of the tumor or administration of substances (N=5); 2) Group Tumor and Copaiba (GTCOP) with animals subjected to the injection of Walker 256 tumor in the bladder and treated with 0,63 mL/kg of copaiba oil by gavage (N=5); Group tumor (GT) with animals subjected to the injection of the tumor but without treatment (N=5). Was analysed the animal weight variation, tumor weight, inhibition potencial (IP) and hystological analysis of the rats' bladder. **Results**: The average weight variation in GT was 16,4g and GTCOP was 35g, but there was no statistically significant difference ($p = 0.37$). The average bladder weight in GT was 0.728 ± 0.33g and GTCOP was $0.449 \pm 0,214$g, with no statistically significant difference ($p = 0.1549$). The potential for inhibition of copaiba oil was - 358%. There was no significant difference in the histological study. **Conclusion**: The copaiba oil by gavage had no effect on Walker 256 tumor inoculated in the rats' bladder, based on the weight of the animal, tumor weight and histologic features. It can be associated with a low dose used in this study. **Financial support** and acknowledgments: **Financial support** from LCE/UEPA. The Ethics Committee in the Use of Animals approved the research, protocol number 24/14. **References**: MARTINI, T. International Braz j urol., v. 39, p; 622, 2013. OGATA, D.C. Rev. Col. Bras. Cir., v. 39, p. 394, 2012. JACOBS, B.L. CA Cancer J. Clin., v. 60, p. 244, 2010. GARCIA, R.F. Rev. Saúde e Pesquisa, v. 5, p. 137, 2012. SOUZA, C.M.P. Rev. bras. Plantas med., v. 15, p. 188, 2013. BRITO, N.M.B. Acta Cir. Bras., v. 25, p. 176, 2010. MORAES, S.P. Acta Cir. Bras., v. 15, p. 252, 2000. DORNELAS, C.A. Acta Cir. Bras., v. 21, p. 38, 2006. TARNOWSKI, G.S. Câncer Res., v. 17, p. 1033, 1957. YAMAGUCHI, M.H. Revista Saúde e Pesquisa, v. 5, p. 137, 2012. GOMES, N.M. J Ethnopharmacol., v. 119, p. 179, 2008. WU, W. Cancer Res., v. 64, p. 1757, 2004. TISDALE, M.J. Curr. Opin. Gastroenterol., v. 26, p. 146, 2010. BOTELHO, N.M. Rev. Para. Med., v. 26, p. 7, 2012. LIMA, S.R. Phytoter Res., v. 17, p. 1048, 2003.

10.013 *In vitro* **cytotoxicity of synthetic hydrazones against human tumor cell lines.** Brito JV[1], Oliveira AC[1], Rocha DD[1], Pessoa CO[2], Sousa NS[3] [1]UFC – Farmacologia UFC, [2]Fiocruz-CE, [3]UFRJ

Introduction: Cancer is an assembled of diseases characterized by uncontrolled growth and spread of abnormal cells. According to INCA (National Institute of Cancer José Gomes Alencar da Silva), in 2016, Brazil are going to have 400 thousands new cases of cancer. Thus, there is a class of organic compounds called hydrazones, which possess a promising in vitro and in vivo cytotoxic activity against tumoral cell lines (Verma et al, 2014). **Methods:** A series of twelve hydrazones derivatives were synthesized and screened for their cytotoxic activity against tumoral cell lines by high throughput screening. Then, the compounds that showed growth inhibition equal or greater 75% compared to controls, were selected to IC_{50} test. To IC_{50} determination, the cell lines tested were NCI-H460 (lung Cancer), HCT-116 (colorectal carcinoma), PC3-M (prostate cancer), MDA-MB-231 (breast cancer), PBMC (primary peripheral blood mononuclear Cells) and L929 (mouse C3H/a connective tissue). Compounds were tested at a range of 0.2-25 µM for 72h and the effect on cell proliferation was evaluated in vitro by the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay, as described by Mosmann. After that, IC_{50} (the concentration that inhibits growth in 50%) values were calculated, along with the respective 95% of confidence interval (CI), by non-linear regression using the software GraphPad Prism 5.0. **Results:** Four of the twelve compounds tested were selected: PM 4, PM 5,PM 9 and PM 10. Compound PM 4 showed the most potent cytotoxic effect against all cell lines tested, with IC_{50} ranging from 0.45 µM to 2.70 µM. However, PM5 showed more selective effect to the tumor cell lines, which NCI-H460 was the most sensitive with IC_{50} of 1.35 µM. Whereas, the IC_{50} values for the nontumoral cells were above 25 µM. In addition, the compounds PM9 and PM10 also demonstrate more selectivity to NCI-H460 similar to PM5, with IC_{50} values of 2.60 and 2.20 µM, respectively. **Conclusion:** In summary, four hydrazones tested showed satisfactory cytotoxic activity on four tumoral cell lines. Even these compounds are all hydrazones, they have little modifications in their radical structure, that allows them to act differently from each other. Furthermore, the most promising one seems to be PM5, because of its selectivity to NCI-H460 cells. Therefore, further studies are needed for understanding the mechanisms of action of PM5. **Financial support:** The governmental Brazilian agencies CNPq , CAPES and FUNCAP. **Reference:** Verma, G., Marella, A., Shaquiquzzaman, M., Akhtar, M., Ali, M. R., & Alam, M. M. (2014). A review exploring biological activities of hydrazones. *Journal of Pharmacy & Bioallied Sciences*, *6*(2), 69–80. http://doi.org/10.4103/0975-7406.129170 BRASIL. Instituto Nacional de Câncer José Alencar Gomes da Silva (inca). Ministério da Saúde. Estimativa 2016: Incidência de Câncer no Brasil. Rio de Janeiro: Serviço de Edição e Informação Técnico-científica, 2015. 121 p. Disponível em: <http://www.inca.gov.br/estimativa/2016/estimativa-2016-v11.pdf>. Acessed in 31/ may / 2016.
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10.014 Melatonergic system modulates human medulloblastoma cell growth and patient survival. Ostrowski LH[1], Kinker GS[1], Marie SNK[2], Rivara S[3], Spadoni G[4], Markus RP[1], Fernandes PA[1] [1]IB-USP – Fisiologia, [2]FM-USP – Neurologia, [3]Università degli Studi di Parma – Farmacologia, [4]Università degli Studi di Urbino "Carlos Bo" – Química Farmacêutica e Toxicológica

Introduction: Medulloblastoma, observed in the cerebellum or in the fourth ventricle, is the most common central nervous system tumor in children. The synthesis of melatonin by human gliomas reduces cell line growth and increases patient survival (Kinker *et al*., J Pineal Res, 60, 84, 2016). Melatonin exerts systemic and local protective roles through the activation of GPCRs melatonin receptors (MTRs; MT1 and MT2). Considering the well-known protective role of local melatonin in the cerebellum (Pinato *et al*. Brain Struct Funct, 220, 827, 2015), here we evaluated whether activation of MTRs by medulloblastoma synthesized melatonin improves patient survival. **Methods:** We determined the basal production of melatonin by DAOY human medulloblastoma cell line after 6 hours of culture. We also analyzed the immune-like expression of arylalkylamine N-acetyltransferase (AANAT), acetylserotonin O-methyltransferase (ASMT), MT1 and MT2 receptors. Cell growth after 48 h incubation with melatonin (10^{-11} - 10^{-5} M), 2-iodomelatonin (10^{-12} - 10^{-6} M), DH97 (10^{-9} - 10^{-6} M) or 5-HEAT (10^{-9} - 10^{-6} M) was determined by MTT assay. These data, normalized by the respective vehicle, are presented as mean ± SEM of 3 independent experiments; $p<0.05$ was considered significantly different. Finally, using a database of human medulloblastoma patients (Cho *et al*., J Clin Oncol, 29, 1424, 2011), we analyzed the gene expression of *AANAT*, *ASMT*, *CYP1B1* (degradation of melatonin in brain tissues), *MTNR1A* (MT1) and *MTNR1B* (MT2). The prognostic value of the index designed to predict melatonin content in tumor biophase (*ASMT:CYP1B1*) was evaluated. **Results:** DAOY production of melatonin was confirmed by the immune-like expression of AANAT, ASMT and the detection of 10 ± 0.7 pg/mL (n = 6) of melatonin in the culture medium. The inhibitory effect of melatonin (20%) and 2-iodomelatonin (40%) on DAOY growth, as well as, the expression of both MTRs suggests a modulatory role for tumor produced melatonin. The selective antagonist to MT2 receptor, DH97, used for evaluating endogenous melatonin effect showed an inhibition of cell growth (25%) at low concentrations, and a dose-dependent return to initial levels with higher concentrations that inhibits both MT2 and MT1 receptors. Therefore, it was supposed that melatonin acts on both receptors resulting on opposite effects. Accordingly, 5-HEAT, a MT1 agonist and MT2 antagonist induced a stronger inhibition of DAOY growth (75%). Bioinformatics analysis showed a reduction in the expression of *MTNR1A* gene in medulloblastoma patients and the *ASMT:CYP1B1* index as a positive independent prognostic factor regarding survival (hazard ratio, HR: 2.91, p = 0.046) and tumor recurrence (HR: 3.53, p = 0.008). **Conclusion:** Our data indicate melatonin receptors as opposite modulators of human medulloblastoma and that tumor production of melatonin is a positive prognostic factor. Therefore, this new approach for studding medulloblastoma opens new targets for pharmacological investigation. **Financial support:** FAPESP (2013/13691-1, 2014/23830-1, 2015/23348-8).

10.015 Effects of ML3403, a P38/MAPK inhibitor, on human glioma cell proliferation. Marchi FO, Tort ABL, Laufer S, Cappelari AR[1], Morrone FB PUCRS

Gliomas are primary tumors of the central nervous system that are associated with a high mortality rate. P38/MAPK is part of the mitogen-activated protein kinases (MAPK) family (JOVCEVSKA I. Rev. Mol Clin Oncol, v. 1, p. 935, 2013). There are four isoforms identified for their well-known functions, among these, is the control of inflammation (CUADRADO A. Rev. Biochem J, v. 429, p. 403, 2010). Recent studies indicate that chronic inflammation can promote some types of cancers, including gliomas (HUANG P. Rev. Protein Cell, v. 1, p. 218, 2010). The goal of the present study was to investigate the effects of ML3403, an inhibitor of p38/MAPK, on the viability of glioma cells, and to assess its effect when combined with radiotherapy. **Methods:** The human glioma cell lines, U251 and U138, were cultured in DMEM supplemented with 10% of fetal bovine serum (FBS) at a temperature of 37ºC, a minimum relative humidity of 95%, and an atmosphere of 5% CO2 in air. The cell viability test was performed using the MTT method, where the cells were treated with ML3403 (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 150 and 200 µM) for 24 hours. Cell counting was also performed to examine the proliferation, the cells were treated with ML3403 concentrations (10, 20, 50, 100, 150 and 200 µM) for 24 h and then counted in Countess FL II apparatus. To evaluate the effect of the compound ML3403 in association with radiotherapy, the cells were treated with ML3403 concentrations of 10. 20, 50, 100, 150 and 200 µM and after were irradiated with a dose of 2 Gy, the viability assay was performed by MTT. Data were analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test, p values <0.05 were taken to indicate statistical significance. **Results:** In the MTT assay we observed a significant decrease in cell viability at a concentration of 200 µM for the U138 line (24.1 ± 3.2%), whereas for U251 line was not observed significant decrease in any of the tested concentrations. When associated with radiotherapy, it was observed a decrease of U251 cell viability at concentrations of 100, 150 or 200µM (26.7 ± 7.9, 33.4 ± 7.9% and 49.6 ± 9.9%, respectively) and U138 line decreased cell viability only at the concentration of 200 µM. In the proliferation assay, by cell count, ML3403 treatment at 150 or 200 µM decreased significantly U251 cell number (68.6% ± 6.8% and 74.5% ± 7%, respectively) and ML3403 treatment at 100, 150 or 200 µM reduced U138 cell number in relation to control cells (32.7% ± 6%, 34.7% ± 5.9% and 34.6% ± 5.9% respectively). C**onclusion:** It is known that the mechanism of the compound ML3403 and the MAPKs pathway are implicated in the development/growth of tumors. In this study, the combination of ML3403 with radiation therapy seems to contribute synergistically to the action of ML3403. Although, more experiments are needed to help in understanding how this compound in combination with radiotherapy acts in glioma cells. This work was financially supported by PUCRS, FAPERGS.

10.016 Copaiba oil effect on Walker 256 tumor evolution in female rats kidney. Aguiar MMF, Nunes MP, Praia WC, Nascimento JBL, Dórea MA, Leite GMO, Gonçalves BH UEPA – Cirurgia

Introduction: Kidney cancer is the third most frequent of genitourinary system and represents approximately 3% of malignant adult diseases. It is also known as Renal Hypernephroma or Adenocarcinoma, the most common form is clear cell type, accounting for 85% of diagnosed tumors. Individuals between 50 and 70 years are considered a risk group for kidney cancer development. The aim of this study is evaluate the effects of Copaiba oil (Copaifera reticulata) on inoculated Walker 256 tumor in female rats kidney. **Methods**: The Ethics Committee in the Use of Animals of the State University of Para (UEPA) approved the research. Twenty female mice were used, weighing between 180-230 g. The animals were randomized distributed into four groups: 1) Normality – the animals were not submitted to Walker 256 tumor inoculation or Copaiba oil administration (N=5); 2) Tumor + Copaiba- Walker 256 tumor inoculated in kidney and subsequent Copaiba oil administration by gavage (0,63 ml/Kg) (N=5); 3) Tumor – Walker 256 tumor inoculated without oil administration (N=5) and 4) Pilot group- surgical simulation (N=5). The animals were observed for ten days, on the eleventh day the animals were euthanized. After the euthanasia was confirmed, the kidneys were collected for the histopathological analysis. The Tarnowisk and Stock formula was used to evaluate the inhibitory potential of Copaiba oil (Copaifera reticulata). It was adopted 5% as the level of significance. **Results**: The application of Tarnowisk and Stock formula resulted in 32% ($p<0,05$) inhibition of tumor growth in Tumor + Copaiba group. There was no difference between Tumor and Tumor + Copaiba groups, in variation of tumor weight according with the analyzed parameters ($p>0.05$). **Conclusion**: In conclusion, Copaiba oil (Copaifera reticulata) has an inhibitory effect on Walker 256 tumor growth but does not attenuate weight loss resulting from the tumor. **Financial support** and acknowledgements: **Financial support** from LCE/UEPA. The Ethics Committee in the Use of Animals approved the research, protocol 27/14.

10.017 Chemical characterization of the Copaiba's oil essence and cell viability study. Santos JM, Souza VB, Radaic A, Mazon SB, Queiroga C, Schenk AA, Cunha IBS, Marques LA, Eberlim MN

Introduction: The genus *Copaifera sp* (*Leguminosae – Caesalpinioideae*), also known as "Copaiba", Copaibeiras", "Pau d'óleo", that naturally grows in Africa (4spp.), Central America and South America presents important pharmacological properties. Even with the already proved scientific applications, like the diuretic action, antiseptic action of urinal system, healing and anti-inflammatory results, and a tumor inhibitor, there are still contradictions related to the variation of physico-chemical characteristics of the Copaiba Oil. In this context, it was analysed, using comparative effects, the chemical characteristics of 3 types of resin-oils and their respective antineoplasics effects against the cell line of the human glioma (U251). **Method:** The composition analysis of the copaiba oils 1 (Pernambuco samples); 2 (Manaus sample) and 3 (Manaus sample), was analyzed using two assays, the first by a chromatography system coupled to gas Agilent mass spectrometer (HP6890) equipped with HP5MS column. The second assay it´s by right infusion, in negative mode, and electrospray ionization (ESI [-] - MS) using automatic chromatograph injector UHPLC coupled with a mass spectrometer Q-TOF (LC-MS-6550 iFunnel Agilent Technology). To analyse the citotoxicity and cell viability, it was used the colorimetric essay of MTT (3 - (4,5-dimethyl-2-yl) -2-5 diphenyltetrazolium bromide). Nine thousand cells/well (cell line of the human glioma - U251) were put on 96-well plates. The cells were treated with copaiba oil using logarythmic concentrations of 100-0, 0001µg/ml (100uL/well). After 72 hours of incubation, the plates were treated with MTT (Sigma, M5665) 5mg/mL dissolved in buffer PBS (Sigma P4417). The absorbance was done using *Plates Reader Sinergy Elisa* (Bio Tek Instruments, Highland Park, Winooski, USA) at 570nm. The experiment was applied on triplicates and the results were presented in average values ± standard deviation. As a positive control, it was used chemotherapic doxorubicin. These studies were approved by the Ethics Research Committee da Universidade Estadual de Campinas, under the protocol 2414-1. **Result:** The obtained specters were based on review studies that gather the main types of compounds within the copaiba oil. Through EASI-Orbitrap, it was found 15 types of compounds within the oils, among them are, 3-clerodeno-15,18-dioic acid, 13-clerodeno-15 acid, copalic acid and 11-acetoxy-copalic acid. In these conditions, the sample from Pernambuco presented citotoxic activity in concentrations of $6{,}171.10^{-2}$ µg/ml, for the Manaus oil (2) $8{,}344.10^{-2}$ µg/ml. Other Manaus sample presented (3) $1{,}385.10^{-4}$ µg/ml about U251 cells. **Conclusion:** The 3 types of resin-oils presented positive influence on cell proliferation, using *in vitro* experiments, existing relation between concentration and effect. Thanks to the CNPq for their Financial Support

10.018 Antineoplastic effects of the soluble fraction of polysaccharide (SFP) from red wine in Walker-256 tumor-bearing rats Stipp MC[1], Corso CR, Livero F[1], Lomba LA[1], Bezerra I[2], Telles JE[3], Cavalieri E[3], Klassen G[3], Sassaki G[2], Acco A[1] [1]UFPR- Farmacologia, [2]UFPR-Bioquímica, [3]UFPR- Patologia

Introduction: Cancer is a malignant disease triggered by normal cells mutation, which incorporate features that promote cell survival beyond its shelf life, giving origin to a disordered cells proliferation. The main treatment for cancer patients is chemotherapy. However, by inducing a lot of side effects, new compounds with less toxicity have been investigated. The polysaccharides are substances that modify biological response without causing local damage, and provide the body adaptation to several biological stresses (WASSER, 2003). Therefore, the aim this study is to investigate the antitumor activity of soluble fraction of polysaccharides (SFP), extracted from cabernet franc wine, in the carcinossarcoma model Walker–256 in rats. **Methods**: Male Wistar rats received 30 or 60 $mg.kg^{-1}$ SFP, or vehicle, orally, once a day, for 14 days after subcutaneous inoculation of $2x10^6$ Walker-256 tumor cells. The tumor development was daily monitored and in the end of the treatment the animals were anesthetized for biological material collection. Blood count and plasma biochemistry were performed, besides measurements of inflammatory parameters and gene expression in tumor tissue. All the experimental protocols were approved by the Ethical Committee for Animal Use (CEUA) of Biological Sciences Section of UFPR (№ 908). **Results**: Both doses of SFP reduced significantly the tumor weight and volume compared with the control (vehicle). The treatment with 60 $mg.kg^{-1}$ SFP modulated the immune and inflammatory response, by reducing the blood monocytes and neutrophils, and increasing the lymphocytes number. Also, the treatment reduced the tumor activity of NAG, MPO and NO, and increased the tumor TNF-α level. The tumor histology with HE showed high degree of necrosis in animals treated with SFP. In consonance, the treatment induced the gene expression of *RIP1* and *RIP3*, both related with necroptosis pathway, but did not change the genes of apoptosis via, namely *p53, Bax, Bcl-2* and *caspase-3*. **Conclusion**: The SFP showed antineoplastic activity against the solid tumor Walker-256 in rats. This effect is due to its action on immunologic system, controlling the tumor microambient and stimulating the TNF-α production, which may trigger the necroptosis pathway. **Financial support**: CAPES, CNPq. **Reference:** WASSER, S. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, v. 60, n. 3, p. 258–274, 2003.

10.019 Estrogen receptor mediates the regulation of the N-Cadherin in androgen-independent prostate cancer cell PC-3. Silva RS, Lombardi APG, Porto CS Unifesp-EPM – Farmacologia

Introduction: The activation of multiple signaling pathways is involved in castration-resistant prostate cancer (CRPC), including Wnt/β-catenin pathway. Direct evidence of crosstalk between Wnt and estrogen signaling pathways via functional interaction between β-catenin and ESR1 (ERalpha) has been shown in different cells (reviewed in Lombardi et al., Spermatogenesis 3:e23181, 2013). In androgen-independent prostate cancer cell PC-3, used *in vitro* and in xenograft implants as CRPC models, the activation of ESR2 (ERβ) by 17beta-estradiol (E2) or ESR2-selective agonist DPN increased the non-phosphorylated β-catenin expression. Furthermore, E2 plays a role in PC-3 cell proliferation through a novel pathway, involving ESR2-mediated activation of beta-catenin (Lombardi et al., Mol Cell Endocrinol 430:12, 2016). N-cadherin is connected via alpha-catenin and beta-catenin to the cytoskeleton and functions as both a mechanical cell adhesion component and a signaling molecule. This protein has emerged as an important oncology therapeutic target; it is involved in regulating the proliferation, survival, invasiveness and metastasis of cancer cells (reviewed in Blaschuk, Phil. Trans. R. Soc. B 370:20140039, 2015). Thus, this study was performed to investigate the effects of the activation of the estrogen receptors on N-cadherin expression in PC-3 cells. **Methods:** The experimental procedures were approved by the Research Ethical Committee at Escola Paulista de Medicina-Universidade Federal de São Paulo (5878240314).PC-3, androgen-independent prostate cancer cell, and PNT1A, human post pubertal prostate normal cells, used as a control of some experiments, were grown in RPMI 1640 medium without phenol red, supplemented with 10% of fetal bovine serum, HEPES (5.95 mg/ml) and gentamicin (0.02 mg/ml). After 24 hours of serum removal, PC-3 and PNT1A cells were incubated in the absence (control) and presence of ESR2-selective agonist DPN (10nM) for 10 and 30 minutes, and 1, 2 and 24 hours. Western Blot and immunofluorescence assays were performed as previously described (Lombardi et al., 2016), using rabbit polyclonal antibody anti-N-cadherin (sc-7939, Santa Cruz) and actin (sc-1,616), as a protein loading controls. **Results and Conclusion:** N-cadherin immunostaining was preferentially found in the membrane of the PNT1A cells. In PC-3 cells, a diffuse immunostaining for N-cadherin was detected in the cytoplasm. Treatment of PC-3 cells with DPN for 24 hours markedly decrease N-cadherin expression (50%) by Western Blot analysis. On the other hand, the treatment with DPN did not have any effect in the PNT1A cells. Taken together our results, DPN may induce beta-catenin released from the cell-cell junctions by downregulation of N-cadherin (degradation or inhibition of N-cadherin transcription) and facilitates beta-catenin dependent signaling. **Financial support**: FAPESP and CNPq.

10.020 Role of dermcidin in Zelboraf / Vemurafenib acquired resistance in melanoma cells Montoya JE, Belizário JE ICB-USP – Farmacologia

The dermcidin (DCD) is an protein of 11-kDa produced by epithelial cells of the eccrine glands of the skin and released in sweat where it act as peptide actibiotic. Amplification of DCD locus on chromosome 12, band q13, causes a proliferative advantage and a resistance to cytotoxic drugs inr many types of cancers, including breast and melanoma. This project aims to elucidate the role of DCD in cutaneous melanomas acquired resistance to vemurafenib induced resistance and dependency on on BRAF(V600E)-MEK-ERK resistance using models *in vitro* and *in vivo*. We have applied biochemical and genomic tools and cell line G361 with BRAF V600E mutated gene and identified that overexpression of DCD is correlated with resistance to vemurafenib in in vitro assays In in vivo assay we observed that G-361 xenografet tumors are larger and induce cancer cachexia at 20-30 after implantation. The study of cooperation between the DCD and the BRAF pathway we will test the strategy to inhibit the expression of DCD through use of RNAi or polyclonal antibody. We have treated mice with G-361-DCD and DCD-knockout tumors with vemurafenib 50 mg/kg. We will select clones G361 cell clones and applied transcriptome sequencing of resistant clones to search fo genetic variations associated with resistance and the expression of proteins of interest obtained by immunohistochemical assays, Western blot to explore mechanisms and pathways of resistance between the selected clones. Thus, we expect to identify new therapeutic targets which could be of use in combination therapy in the treatment of resistant melanoma.Support from CAPES, CNPq and FAPESP.

10.021 Modulation assessment of purinergic receptor P2Y12 BY clopidogrel in glioma cells Vargas P[1], Cappellari AR[1], Corte T[3], Ferreira J[1], Kunde M[1], Morrone F[2] [1]PUCRS – Farmacologia Bioquímica e Celular, [2]PUCRS – Farmacologia Bioquímica e Celular, [4]PUCRS – Ciências Farmacêuticas

Introduction: Gliomas comprise tumors of glial cells or neuroglia that can be classified by stages I-IV (Goodenberger, *Cancer Genetics*, v. 205, n. 12, p. 613, 2012). The additional classification is grade determined by histopathology, the presence of polymorphism nuclear and necrosis (Nicoletti,*Journal of Neuro-oncology*, v. 120, n. 2, p. 235, 2014). Glioblastoma (GMB) is the most aggressive primary tumors in central nervous system (Meijer, *Cancer Gene Therapy, v. 16, n. 8, p. 664*) and continues to rank among the most lethal tumors (Noch et al., *Cancer Biology & Therapy*, v. 8, n. 19, p. 1791, 2009). The purinergic system is involved in various neuronal and non-neuronal mechanisms, such as, immune responses, inflammation, platelet aggregation, and may lead to proliferation and cell death. ATP is a purinergic signaling molecule, existing in all cells and is involved in the regulation of several pathophysiological processes (Burnstock, *Current Opinion in Pharmacology*, v. 4, n. 1, p. 47, 2004). The P2Y12 is considered a chemoreceptor for ADP (Dorsam, *Journal of Clinical Investigation*, v. 113, n. 3, p. 340, 2004). Its expression is documented in some cancer types, such as the rat glioma C6 cell line, renal carcinoma and colon carcinoma. However, its role in the development of signaling and change the chemotherapy is not well-elucidated (Sarangi, *Medical Oncology*, v. 30, n. 2, p. 1, 2013). Clopidogrel bisulfate is an antiplatele drug (Jiang, *European Journal of Pharmaceutical Sciences*, v.82 p. 64, 2015), which inhibits irreversibly the P2Y12 receptor (Brown, *Journal of the American Academy of Dermatology*, v. 72, n. 3, p. 524, 2015). OBJECTIVE: To evaluate the effect of clopidogrel in glioma cell death, viability and proliferation. **Methods**: Cell Viability: After treating the C6 cells with 150 µM or 300 µM of a clopidogrel aqueous solution for 24 h, the MTT assay was accomplished (n=4). Cell count: Rat glioma C6 cells were treated with 150 µM or 300 µM of a clopidogrel aqueous solution for 24 h (n=3), the cell count was performed using trypan blue and an automatic cell counter. Clonogenic Assay: The cells were trated with 150µM and 300µM for 24h. The remeaning cells were kept in culture for 7 days, then stained with gentian violet and counted (n=5). Statistical comparison performed by one-way ANOVA followed by Tukey's test, and $p<0.05$, and T-test with $p<0.05$ was considered as significant. **Results**: The glioma C6 cells that was exposed to 150 µM and 300 µM for 24 h showed a significant reduction in viability (150 µM or 300 µM) (68.63 ± 8.97; 55.06 ± 13.70, respectively), and proliferation (300 µM) (23.92 ± 14.31) when compared to control. The clonogenic assay was performed for 7 days with the cells previously treated with clopidogrel bisulfate, proving that the treatment was effective to reduce they capacity of division in the remain cells (11.00 ± 3.22). **Conclusion**: Our results have shown that clopidogrel bisulfate treatment caused glioma cell death, diminished cell viability, proliferation and recurrence. **Financial Support**: CAPES and CNPq.

10.022 Evaluation of the synergistic effect of sodyum butyrate and tyrphostin AG1478 in glioblastoma cell lines proliferation Buendia M[1], Thomaz A[1], Pinheiro KV[1], Brunetto AL[2], de Farias CB[2], Roesler R[3] [1]UFRGS, [2]Instituto do Câncer Infantil, [3]UFRGS – Farmacologia

Introduction: Gliomas are the most frequent brain tumors, in near of 80% of patients with Central Nervous system (NCS) malignancies, being the Glioblastoma Multiforme (GBM-grade IV) the most aggressive and lethal of all. Among the main features of this type of cancer are resistance to apoptosis, a high mitotic rate, diffuse infiltration, a tendency for necrosis, significant angiogenesis, and genomic aberrations. Despite current multimodality treatment efforts including maximal surgical resection if feasible, followed by a combination of radiotherapy and/or chemotherapy, the prognosis for GBM patients remains poor and the median survival after diagnosis is about 14 months. New diagnostic and therapeutic strategies that target these pathways to improve the treatment of malignant glioma are needed. Combination of therapies with synergistic effects in the cellular signaling pathways of cancer could potentiate the anti-tumor effect of monotherapy alone. **Methods**: U87 and A172 cell lines were treated with the anti-EGFR Thyrphostin AG1478, the Histone deacetylase inhibitor (HDACi) Sodyum Butyrate (NaB), respectively or combination of both, for 72 hours. The cellular proliferation was measured through the trypan-blue assay on neubauer chamber. **Results**: We found that both, AG1478 as NaB, are able to reduce the proliferation of both cell lines studied and, to date, our study has shown that the combination of drugs in a therapeutic dose is able to slightly enhance the effect of monotherapies. **Conclusion**: Our studies show that inhibition of epidermal growth factor receptor, in conjunction with the inhibition of the histone deacetilases leads to greater reduction of proliferation in cultured of lineages of GBM. Further studies should be performed to discover if there is interaction between the signaling pathways of both targets. **References**: Cory A, Okezie O.K, Ankit I.M, Chunhui D, Ningjing L , Austin K.M, Darell D.B. (2009). Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opinion on Investigational Drugs. 18(8). Gurney J.G, Kadan-Lottick N (2001) Brain and other central nervous system tumors: rates, trends, and epidemiology. Current Opinion Oncology. 13:160-166. Raizer J.J (2005). HER1/EGFR tyrosine kinase inhibitors for the treatment of glioblastoma multiforme. Journal of Neuro-Oncology. 74: 77–86. Schwartzbaum J.A., Fisher J.L., Aldape K.D. & Wrensch M. (2006). Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol. 2(9), 494-503. Zahonero C, Sanchez-Gomez P. (2014) EGFR-dependent mechanism in glioblastoma: towards a better therapeutic strategy. Cell. Mol. Life Sci. 71:3465–3488. **Financial support**: - ICI-RS – CNPq - FIPE / HCPA CEP/HCPA: 16-0204.

10.023 Synergistic activity of deguelin and fludarabine in cells from chronic lymphocytic leukemia patients and in the New Zealand black murine model. Rebolleda N[1], Losada-Fernández I[1], Perez-Chacon G[2], Castejon R[3], Rosado S[3], Morado M[4], Vallejo-Cremades MT[5], Martinez A[1], Perez-Aciego P[1], Vargas JA[3] [1]Fundación LAIR, Madrid, Spain, [2]Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, Madrid, Spain, [3]Servicio de Medicina Interna, Hospital Universitario Puerta de Hierro Majadahonda, IDIPHIM, Universidad Autónoma de Madrid, [4]Servicio de Hematología y Hemoterapia, Hospital Universitario La Paz, Madrid, Spain, [5]Laboratorio de Imagen, Plataforma Apoyo a la Investigación, IdiPaz, Hospital Universitario La Paz.

Introduction: B-cell chronic lymphocytic leukemia (CLL) remains an incurable disease, and despite the improvement achieved by therapeutic regimes developed over the last years still a subset of patients face a rather poor prognosis and will eventually relapse and become refractory to therapy. The natural rotenoid deguelin has been shown to induce apoptosis in several cancer cells and cell lines, including primary human CLL cells, and to act as a chemopreventiv agent in animal models of induced carcinogenesis. **Methods:** Peripheral blood was collected from 35 CLL patients and 10 healthy control donors in 10 ml heparinized tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation (Lymphocytes Isolation Solution, Comercial Rafer). All patients had been untreated at least for 3 months before blood collection. An experimental model of CLL was established in the mouse NZB strain by transfer of cells from enlarged spleens of aged (>1 year) NZB females into young (4–6 week) female NZB recipients. Spleen cells from three aged diseased female NZB mice were pooled, and 20 x 106 cells per mice were transplanted intraperitoneally (i.p.) into 40 young NZB recipients. Two months after transplants, growth of transplanted leukemic cells was assessed by detection of hyperdiploid B220low CD5low IgM+ cells in the peripheral blood by flow cytometry. The enlarged spleen from one of the transplanted mice was subsequently transplanted i.p. into five young NZB mice used to study the effect of deguelin on CLL-like cells in vivo. Drug treatments in mice consisted on 4 mg/kg deguelin dissolved in corn oil given intragastrically (i.g.) and/or 35 mg/kg fludarabine in saline phosphate (Hospira Pharmaceuticals) given intraperitoneally (i.p.). **Results and Conclusion:** In this work, we show that deguelin induces apoptosis in vitro in primary human CLL cells and in CLL-like cells from the New Zealand Black (NZB) mouse strain. In both of them, deguelin dowregulates AKT, NFκB and several downstream antiapoptotic proteins (XIAP, cIAP, BCL2, BCL-XL and survivin), activating the mitochondrial pathway of apoptosis. Moreover, deguelin inhibits stromal cell-mediated c-Myc upregulation and resistance to fludarabine, increasing fludarabine induced DNA damage. We further show that deguelin has activity in vivo against NZB CLLlike cells in an experimental model of CLL in young NZB mice transplanted with spleen cells from aged NZB mice with lymphoproliferation. Moreover, the combination of deguelin and fludarabine in this model prolonged the survival of transplanted mice at doses of both compounds that were ineffective when administered individually. These results suggest deguelin could have potential for the treatment of human CLL. **Financial support:** This work was supported by Fondo de Investigaciones Sanitarias, Ministerio de Sanidad), PI08/1099 and PI13/01607; La Caixa P22664; and Fundación LAIR P160105.

10.024 Lipoxin A_4 analog selectively alters the tumor-associated macrophage profile leading to control of tumor progression. Simões RL[1], de Brito NM[1], Cunha-da-Costa H[1], Morandi V[1], Fierro IM[1], Roitt IM[2], Barja-Fidalgo TC[1] [1]UERJ , [2]Middlesex University – London, UK

In tumor microenvironments, pro-inflammatory macrophages (M1) acquire anti-inflammatory and pro-tumor characteristics. These tumor-associated macrophages (TAMs), often referred to as myeloid suppressors, exhibit an M2-like profile, with low cytotoxic properties and a deficient modulation of NO and ROS production. Lipoxins (LX) and 15-epi-lipoxins are lipid mediators inducing anti-inflammatory and pro-resolution activities in mononuclear cells, but their effects on TAMs remain to be elucidated. This study tested the hypothesis that ATL-1, a synthetic analog of 15-epi-lipoxin A_4, could modulate the TAM activity and was performed according to guidelines of Ethical committee (CEUA/077/2012/, UERJ). It was shown that human macrophages (MΦ) differentiated into TAMs after incubation with conditioned medium from MV3, a human melanoma lineage cell, presumably by simulating a tumor microenvironment. In contrast with the effects observed,in the other M2 subset and M1 profile macrophages, ATL-1 selectively decreased M2 surface markers in these TAM, suggesting unique behaviour of the M2d subset. The effect was dependent on VEGF signaling and importantly, reproduced by the natural lipoxins, LXA and 15-epi-LXA_4. In parallel, ATL-1 stimulated TAM to produce NO by increasing the iNOS/arginase ratio and activated NADPH oxidase, triggering ROS production. These alterations in TAM profile induced by ATL-1 led to the loss of the anti-apoptotic effects of TAMs on melanoma cells and increased their cytotoxic properties. Furthermore, in addition to reversing the TAM anti-apoptotic effect on MV3, ATL-1 inhibited endothelial cell tubulogenesis activated by TAM, a crucial step in the angiogenic process. Finally, ATL-1 was found to inhibit tumor progression in a murine model*in vivo*, which was accompanied by alterations in TAM profile and diminished angiogenesis. Together, the results suggest unexpectedly that the aspirin-induced lipoxinanalogue down-modulates the tumor progression stimulated by TAM probably by inducing a change in the TAMs from an M2- to an M1-like profile thereby triggering tumor cell apoptosis.

10.025 Dysregulation of redox enzymes in Barrett's oesophagus and gastro-intestinal cancer. Simpson L[1], Battle DM[1], Dias-Gunasekara S[1], Viswanath YKS[2], Benham AM[1] [1]Durham University – Biological and Biomedical Sciences, [2]James Cook University Hospital

Oesophageal cancer is the eighth most frequent cancer in the world, with ~500,000 new diagnoses per year, and is particularly prevalent in the less developed countries of Asia and Africa. Cancers of the stomach and oesophagus (upper gastro-intestinal [GI] system) also have a high mortality rate. One of the major indications for upper GI cancer is Barrett's epithelium. Barrett's epithelium develops inappropriately in the oesophagus, often after oesophageal exposure to stomach acid during reflux. However, how Barrett's epithelium changes from a benign to malignant state is not fully understood. To address whether redox signalling is involved in gastro-intestinal disease, we have examined the function and expression patterns of ER redox enzymes (oxidoreductases and disulphide isomerases) and chaperone proteins in Barrett's epithelium, as well as in cultured oesophageal cell lines. These chaperones and redox enzymes are involved in controlling protein folding and disulphide bond formation and are therefore key to protein secretion, metastasis, angiogenesis and the modulation of the redox environment. Our findings suggest that the endoplasmic reticulum (ER) oxidoreductase Ero1alpha and other members of the Protein Disulfide Isomerase family are upregulated during the transition from Barrett's oesophagus to gastrointestinal cancer. Coupled with evidence that ER resident glutathione peroxidases are downregulated in Barrett's, the data suggests that dysregulation of ER redox networks may be involved in generating oxidative stress that contributes to the development of gastrointestinal cancer. Further exploration of ER chaperone and redox enzyme function in the GI tract will help us to evaluate the potential of these proteins as pharmacological targets in gastrointestinal disease