10. **Cancer and Cell Proliferation**

10.001 Cytotoxic activity of thiophene derivative on pancreatic carcinoma cell line (Panc). Carvalho MS[1], Rocha HAO[2], Moura RO[3], Mendonça FJB[3], Aguiar ACV[1] [1]UFRN – Biofísica e Farmacologia, [2]UFRN – Bioquímica, [3]UEPB – Ciências Biológicas

Introduction: Pancreatic cancer is one of the deadliest of human cancers, usually diagnosed late with a biological phenotype characterized by resistance to all cancer treatment modalities and early metastasis[1]. In the last century, the development of cytotoxic agents was revolutionary for cancer therapy, offered a clear survival benefit but also to offer a better quality of life with reasonable symptom relief[2]. Thiophene nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics[3]. The compounds show variable magnitudes of biological activities such as antimalarial[4],anti-inflammatory[5], analgesic[5], antimicrobial[6] and anticancer[7].This latter property was evaluated in this study through the activity of derivative **7CN-09** of series 2 - (substituted amino) -4,5-dialkyl-thiophene-3-carbonitrile. **Methods:** The derivative was synthesized and tested on the human cancer cell line **PANC** (pancreatic carcinoma). For comparative purposes we used an anticancer drug used in the clinic, doxorubicin (Dox), as positive control. The citotoxicity was evaluated by the MTT test[8]. The compound previously dissolved in DMSO were diluted in DMEM to obtain the final concentrations (5, 10, 25 and 50 μM) and added in 96-well microplates (100μL/well), with a cellular density of 5x10³ cells/well. The cells were incubated in the presence of **7C-N09** by 24, 48 and 72h at 37°C and 5% CO_2, in triplicate. After each period, the supernatant was aspirated, was added 100μL of MTT (5 mg/ml in DMEM) and the microplates were replaced in an incubator for 4h. After that, the supernatant was aspirated and the formazan product was solubilized by the addiction of 100μL of ethanol. The plates were agitated during 15 minutes and the absorbance was read on spectrophotometer at a wavelength of 570nm [5]. Statistical analysis was performed according to their means and their standard errors and deviations were made from non-linear regression using GraphPad Prism (version 6). **Results and Discussions:** The derivative **7CN-09** at a concentration of 5 μM at the first 24h showed 30% of cellular proliferation in comparison with 90,5% of the antineoplasic drug Dox, in the **PANC** cell line. The derivative **7CN-09** showed higher cytotoxic effect at the same concentration and time, compared to standard anticancer drug doxorubicin (Dox). Such derivative demonstrates potential for further preclinical investigations and application to anticancer drug. **Conclusions**: The derivative of thiophene **7CN-09** showed a significant cytotoxic activity which was even higher than that of reference drug Doxorubicin, proved to be a promising anticancer drug, requiring further investigations on its cytotoxic mechanism. **References:** 1. **Spalding D.** Medicine **35**:325. 2007. 2. **Ismael G. F.V.** Cancer Treat Rev **34**:81. 2008. 3. **Jha K.K.** J Pharmacy Res **5**:560. 2012. 4. **Caridha D.** Bioorg Med Chem **20**:3863. 2010. 5. **Sondhi S. M.** Med Chem **4**:146. 2008. 6. **Kashyap V K**. J Antimicrob Chemoter **67**:1188. 2012. 7. **Zaky R. R.** Res J Pharma Bio Chem Sci **2:**757. 2011. 8. **Mosmann T**. J. Immunol. Methods **65**:55. 1983. **Financial Agencies and acknowledgments:** Laboratório de Farmacologia/UFRN Laboratório de Biotecnologia de Polímeros Naturais (BIOPOL)/UFRN Laboratório de Síntese e Vetorização de Moléculas/UEPB. CNPq

10.002 Evaluation cytotoxic of thiophene derivative in PANC and PC3 cells. Aguiar ACV[1], Câmara RBG[1], Rocha HAO[1], Mendonça Junior FJB[2], Moura RO[2], Carvalho MS[3] [1]UFRN – Bioquímica, [2]UEPB – Ciências Biológicas, [3]UFRN – Biofísica e Farmacologia

Introduction: Thiophene belongs to a class of heterocyclic compounds whose structure can be found in certain natural products and is also incorporated in several pharmacologically active compounds[1]. In medicinal chemistry, their derivatives have been very well known for their therapeutic applications such as antimicrobial[2], antiinflamatory[3], antidiabetic[4], antiparasitic[5] and anticancer[6]. This latter property was evaluated in this study through the activity of derivative **6CN-09** of serie 2-(substituted amino)-4,5-dialkyl-thiophene-3-carbonitrile. **Methods:** The derivative was synthesized and tested on the human cancer cell lines: **PANC** (pancreatic carcinoma) and **PC3** (prostate carcinoma). For comparative purposes we used an anticancer drug used in the clinic, doxorubicin (Dox), as positive control. The citotoxicity was evaluated by the MTT test[7]. The derivative previously dissolved in DMSO were diluted in DMEM or RPMI to obtain the final concentrations (5,10, 25 and 50 μM) and added in 96-well microplates (100μL/well), with a cellular density of 5x10³ cells/well. The cells were incubated in the presence of derivative **6CN-09** by 24, 48 and 72h at 37°C and 5% CO_2, in triplicate. After each period, the supernatant was aspirated, was added 100μL of MTT (5 mg/ml in DMEM, except for PC3 cells where was used RPMI) and the microplates were replaced in an incubator for 4h. After that, the supernatant was aspirated and the formazan product was solubilized by the addiction of 100μL of ethanol. The plates were agitated during 15 minutes and the absorbance was read on spectrophotometer at a wavelength of 570nm [5]. Statistical analysis was performed according to their means and their standard errors and deviations were made from non-linear regression using GraphPad Prism (version 6). **Results and Discussions:** The derivative **6CN-09** in the **PANC** cell line at a concentration of 5 μM and at the first 24h presented 67,5% of cellular proliferation in comparison with 90,5% of the antineoplasic drug Dox, demonstrated greater cytotoxic efficacy compared to standard anticancer drug doxorubicin in the same concentration and time. For the **PC3** cell the derivative showed a close effect to the Dox. Different cytotoxic effects shown for different cells line of **PANC** and **PC3** occurred probably because the constitutive and peculiar characteristics of each cellular type and/or due to different pathways of action on the metabolism of these cells. **Conclusions**: The derivative of thiophene **6CN-09** was more selective for the pancreatic carcinoma cell line (**PANC)**, and demonstrated to be more effective than the doxorubicin and promising anticancer drug, requiring further investigations on its cytotoxic mechanism. **References**: **1.**Mishra R. Der Pharma Chemica **3**:38. 2011. **2.**Bondock S. Eur J Med Chem 45: 3692**.** 2010**. 3.**Giri R. S**.** Bioorg Med Chem 18**:** 2796**.** 2010**. 4.**Raval P. Bioorg Med Chem 21**:** 3103**.** 2011**. 5.**Mohareb R.M. Eur Chem Bull 2**: 545.** 2013**. 6.**Romagnoli R**.** Bioorg Med Chem 21**: 2746.** 2011**. 7.**Mosmann T**. J.** Immunol. Methods 65**: 55.** 1983**.** **Financial Agencies and acknowledgments:** BIOPOL/UFRN Laboratório de Farmacologia/UFRN Laboratório de Síntese e Vetorização de Moléculas/UEPB CNPQ

10.003 Evaluation of antitumoral activity of canthinone alkaloid. Torquato HFV[1], Paredes-Gamero EJ[2], Buri MV[2], Ribeiro-Filho AC[2], Martins DTO[1] [1]UFMT – Pharmacology, [2]Unifesp – Biochemistry

Introdution: Cancer is characterized by the accumulation of mutations and genetic disruption of epigenetic mechanisms that confer cancer cells proliferative advantages. Canthinone **(Cant.)** is an alkaloid isolated from *Simaba ferruginea,* a shrub from the Cerrado rhizome. In folk medicine, its infusion is used for treatment of peptic ulcers and wounds. The aim of this study was to evaluate the antitumor potential of **Cant**. in leukemic cell lines *in vitro*. **Methods: Cant.** was purified and isolated from the chloroform fraction obtained from the rhizome of *S. ferruginea* and identified by means of HPLC analyses and compared with pure samples of **Cant.** Cell lines of acute myeloid origin, Kasumi-1 and KG-1 and chronic myeloid leukemic K562 were maintained in culture at 37 ° C and 5% CO_2. Kasumi-1 and K562 were cultured in RPMI medium with 20% and 10% fetal bovine serum (FBS), respectively, and KG-1 was maintained in IMDM medium with 20% FBS. The antitumor activity of **Cant.** was assessed by labeling cells with annexin V-FITC and propidium iodide (PI) which identifies apoptosis and necrosis respectively. 10^5/mL cells were plated and **Cant.** (6.25 to 200 µg/mL) was added to the media and cell viability was assessed 24 h after in flow cytometry. For cell cycle analysis, cells were synchronized by FBS deprivation for 24 h and treated with 10 µg/mL (IC_{50}) of **Cant.** for the same period. DNA was stained with PI and analyzed by flow cytometry. Caspase 3 activity was assessed by staining with the antibody against the active form of caspase 3 by flow cytometry in Kasumi-1 after treatment with 10µg/mL of **Cant.** for 24 h. The antitumor activity was expressed as IC_{50} ± SEM of three independent experiments performed in duplicate and CI_{50}<50 µg/mL values were considered cytotoxic.**Results and Discussion**: IC_{50} (µg/mL) values of **Cant.** against Kasumi-1, KG-1 and K562 cells lines, were 10.7 ± 0.06, 8.6 ± 0.02 and 24.7 ± 0.01 respectively, indicating that the alkaloid was more active in Kasumi-1 and KG-1 cell lines. In cell cycle analysis, percentage of Kasumi-1, KG-1 and K562 untreated and cell lines treated with **Cant.** in the G0/G1 phase were 34.58%, 39.86%, 26.43% and 8.75%, 7.51% and 28.75%, respectively. For in G2/M phase, values were 0.22%, 3.26%, 6.42% for untreated cell lines, and 67.92%, 70.12% and 10.50% for treated group. Treatment with **Cant.** cell lines showed selectivity for acute leukemia in the cell cycle arrest in the G2/M phase. The quantification of caspase-3 activation showed a higher mean fluorescence intensity in the treated cell lines (7.1) in comparison to untreated cells (2.9), indicating that **Cant.** induced cell death by activating apoptotic mechanisms. The antitumor activity of **Cant**. in leukemic cell lines, points to its potential use as a phytochemical for the treatment of hematologic malignancies of myeloid origin. **Financial support/Acknowledgement**: CAPES, CNPq, INAU.

10.004 Evaluation of antitumor activity of the hexane extract of *Calophyllum brasiliense* Camb. Torquato HFV[1], Buri MV[2], Paredes-Gamero EJ[2], Ribeiro-Filho AC[2], Martins DTO[1]
[1]UFMT – Farmacologia, [2]Unifesp – Biochemistry

Introduction: Tumor cells exhibit higher mutation rate compared to normal cells and greater instability in the genes responsible for the maintenance of genomic stability. *Calophyllum brasiliense* is a tree widely distributed throughout the Latin America, occurring in humid regions and swamps. Its stem bark is popularly used in the form of infusion for the treatment of inflammation and chronic ulcers. The aim of this study was to evaluate the antitumor activity of the hexane extract of *C. brasiliense* (HE*Cb*). **Methods:** The stem bark of *C. brasiliense* was cleaned, dried, crushed in a knife mill and the resulting powder was macerated for 7 days with hexane, filtered and concentrated with rotaevaporator. The residual solvent was removed in an oven at 40ºC. Acute myeloid cell lines Kasumi-1 and KG-1 and chronic K562 cells were maintained in culture media. RPMI for Kasumi-1 and K562 with 20% and 10% fetal bovine serum (FBS), respectively, and IMDM for strain KG-1 with 20% FBS, all maintained at 37ºC 3 and 5% CO_2. The antitumor activity was assessed by labeling the cells with annexin V-FITC and propidium iodide (PI) which identify apoptosis and necrosis respectively. 10^5/mL cells were plated and HE*Cb* (6.25 to 200 mg/mL) was added to the media. Cell viability was assessed 24 h after, in flow cytometry and expressed in terms of IC_{50} ± SEM of three independent experiments performed in duplicate IC_{50} values <50µg/mL were considered cytotoxic. **Results and Discussion**: In the Kasumi-1, KG-1 and K562 cell lines IC_{50} (µg/mL) were 135.3 ± 0.03, 65.4 ± 0.03, 79.9 ± 0.02 respectively, and HE*Cb* was inactive against the Kasumi-1 cell lines. These results indicate the potential antitumor activity of the EH*Cb* in leukemic lines. **Financial support:** CAPES, CNPq, INAU

10.005 Mebendazole induces citotoxity, apoptosis and reduce metaloproteinases expression in gastric cancer cell line (AGP-01). Pinto LG[1], Soares BM[1], Barreto LH[1], Assumpção PM[2], Riggins GJ[2], Burbano RMR[1], Montenegro RC[1] [1]UFPA – Citogenética Humana, [2]Johns Hopkins University – Medicine

Introduction: Gastric cancer is the second most common cause of cancer related mortality worldwide (1) mainly due to late diagnosis and therapeutical failure. For this reason, there is an obvious need to develop more efficacious treatment strategies. Mebendazole (MZ) is generally used for the treatment of helmintiasis in humans and animals acting by depolymerizing tubulin and therefore disrupting the functions of microtubules. Recently, MZ has been proved to inhibit the growth of different cancer cells in vitro and in vivo (2,3). **Methods:** The cell viability was determined by 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide assay (MTT) with MZ in a range of 0.3 to 20 μM against AGP-01 cancer cell line. Cell death was analyzed using the differential fluorescent staining with EB/AO. The IF staining was used to analyze tubulin polymerization status and zymography was used to evaluate the role of MMP-2 and MMP-9 in cells treated with MZ. HT1080 cell line was used as control. **Results and Discussion:** MZ significantly inhibited cancer cells growth with an IC_{50} of 0.5891 μM and showed better results when compared with other well-known chemotherapeutic drugs used in clinical as oxaliplatin, irinotecan, etoposideo, paclitaxel, cisplatin displaying 4.769 μM, 41.10 μM, 10.31 μM, 0.6243 μM, 17.23 μM, respectively. MZ induces abnormal spindle formation in cancer cells and in vitro activity was correlated with reduced tubulin polymerization at concentrations tested 0.5 μM and 1.0 μM after 14 hours of treatment. MZ reduced MMP-2 and MMP-9 expression in concentration-dependent manner. Furthermore, MZ induces apoptosis in gastric cancer cell, showing a significantly cellular death at 0.5 μM and 1.0 μM compared to negative control ($p<0.001$). Our results support other studies previously reported that MZ induced depolymerization of tubulin and inhibited normal spindle formation in cancer cells, resulting in mitotic arrest and apoptosis (2). Increased activity of MMPs is associated with stages of tumor growth, invasion and metastasis and is frequently overexpressed in different tumors (2,4). MZ reduced MMP expression in gastric cancer cells. These results support the evidence that MZ can inhibit cell growth and probably, migration and invasion. Furthermore, MZ can be test in clinical trial for patients with advanced gastric cancer, being safe for humans. **Financial Agencies:** CNPQ, CAPES, UFPA, FAPESPA. **References: 1.** Parkin DM, Bray FI, Ferlay J, Pisani P. Global cancer statistics: 2002. CA Cancer J Clin. 2005. 2. Martarelli D, Pompei P, Baldi C, Mazzoni G. Mebendazole inhibits growth of human adrenocortical carcinoma cell lines implanted in nude mice. Cancer Chemother Pharmacol. 2008; 61: 809–817. 3. Bai R-Y, Staedtke V, Aprhys CM, Gallia GL, Riggins GJ. Antiparasitic mebendazole shows survival benefit in 2 preclinical models of glioblastoma multiforme. Neuro-Oncology 2011; 13(9):974–982. 4. Coussens LM, Fingleton B, Matrisian LM. Cancer therapy—matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science. 2002; 295: 2387–2392.

10.006 Study of pterocarpanquinone LQB 118 effects on prostate tumor cell line. Martino T[1], Jordão FC[1], Justo GA[1], Coelho MGP[1], Costa PRR[2], Sabino KCC[1] [1]UERJ – Bioquímica, [2]NPPN-UFRJ

The pterocarpanquinone moiety LQB 118 was shown to be effective against several leukemia cell lines, including MDR phenotype (Maia RC, Invest New Drugs, 29:1143, 2011), with low cytotoxicity against peripheral blood mononuclear cells from healthy donors (Netto CD, Bioorg Med Chem, 18:1610, 2010). Prostate cancer (PCa) is the first leading cause of men death in Brazil and shows low response to chemotherapy treatment due to high drug resistance. As the effects of LQB 118 on prostate cancer cells are unknown, this work studied the *in vitro* antitumor effects of LQB 118 and some mechanisms of action on prostate cancer cells. Non-responsive androgen tumor (PC-3) and non-tumor (RWPE-1) prostate cell lines were cultured in RPMI-1640 medium supplemented with 10% FCS and K-SFM medium supplemented with BPE and Epidermal growth factor, respectively. After overnight adhesion, cells (1×10^5/mL) were treated or not (control) with LQB 118 or Paclitaxel (positive control) for 48 h. Cytotoxicity was determined by the MTT assay, cell cycle and mRNA expression were analyzed by propidium iodide (PI) dye and RT-PCR, respectively. PC-3 cell apoptosis was visualized by annexin V/FITC labeling, fluorescence microscopy with DAPI nuclear dye and internucleosomal DNA fragmentation ladder. PC-3 cells were 2.5 fold ($p<0.001$) more sensitive to LQB 118 cytotoxic effects than to Paclitaxel, at 2.5 µg/mL. Otherwise, RWPE-1 cells were more sensitive to Paclitaxel effects (at 0.01 and 0.1 µg/mL) than to LQB 118, which was not cytotoxic at those concentrations. LQB 118 (5 µg/mL) inhibited ($p<0.001$) PC-3 cells proliferation since 12 h (36%) up to 72 h (92%), arrested cell cycle ($p<0.05$) at S and G_2/M phases (95% and 79%, respectively), compared to control (15.4 $\pm$ 4.2% and 21.4 $\pm$ 3.7%, respectively). Paclitaxel (5 µg/mL) induced similar effects, inhibiting PC-3 cell proliferation and arresting cell cycle ($p<0.001$) at G_2/M phase. LQB 118 reduced ($p<0.001$) c-Myc, cyclin D1 and cyclin B1 mRNA expression of PC-3 cells and was more effective than Paclitaxel on increasing early (380 $\pm$ 1% and 64 $\pm$ 1%) and late apoptosis (329 $\pm$ 4% and 124 $\pm$ 7%) levels, respectively, compared to control culture. LQB 118 also induced more evident internucleosomal DNA fragmentation and chromatin condensation. Concluding, LQB 118 was more cytotoxic than Paclitaxel on prostate cancer cells and less cytotoxic on non-tumor cells, compared to Paclitaxel, and was able to inhibit PC-3 cells proliferation, arresting the cell cycle in S and G_2/M phases. These effects are likely related to downregulation of c-Myc, cyclin D1 and cyclin B1 gene expression modulation, leading to apoptosis cell death. Support: CNPq, FAPERJ, CAPES and UERJ.

10.007 ***In vitro*** **anticancer potential of essential oils from *Piper* species of the Amazon**. Pinto AVU[1], Pinto LC[1], Soares BM[1], Barreto LH[1], da Silva JKR[2], Maia JGS[2], Andrade EHA[3], Burbano RMR[1], Montenegro RC[1] [1]UFPA – Laboratório de Citogenética Humana, [2]UFPA – Química

Introduction: The genus *Piper* is largely distributed in the tropical and subtropical region of the world and has been extensively investigated as the source of new natural products with potential antifungal, antitumoral, antioxidant, antiplasmodial and tripanocidal properties (1). **Methods:** The aerial parts of *Piper hispidum, P. anonifolium* and *P. aleyreanum* were collected in Carajás National Forest, Pará state, Brazil, and their essential oils obtained by hydrodistillation using a Clevenger-type apparatus and analyzed by GC and GC-MS. Essential oils (0.4 to 25 µg/mL) were tested for cytotoxic activity against three cancer cell lines: HCT-116 (colon), SKMEL19 (melanoma), ACP-03 (gastric) compared to the positive control (doxorubicin). Also, *Piper* oils were evaluated for its antioxidant properties by DPPH assay.The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl 2*H*tetrazolium bromide (MTT) and IC50's were calculated by nonlinear regression using Graphpad program. **Results and Discussion:** In total, 87 constituents were identified in the *Piper* oils. The sesquiterpenes, both hydrocarbons and oxygenated were the most highly represented classes, the former ranging from 38.6% to 56.6% and the latter varying from 16.6% to 38.9%. Sesquiterpene hydrocarbons belonging to elemene structure are mainly composed by b-, δ- and g-elemene, where b-elemene is accounting for 60% to 72% of all three isoforms. The *P. aleyreanum* oil showed high cytotoxicity and selectivity to melanoma cell line (IC50: 7.4 µg/mL), whereas the other two *Piper* oils did not display cytotoxicity against all cancer cells treated when compared with the control. In addition, *P. aleyreanum* showed high antioxidant activity (412.2 mg TE/mL). In principle, the significant activity attributed to *P. aleyreanum* oil may be accounted by the presence of the high proportion of b-elemene (16.3%) and δ-elemene (8.2%) in its composition. It has been reported that b-elemene exerts anti-cancer potential in brain, laryngeal, lung, breast, prostate, cervical, colon and ovarian carcinomas (2-3). Our results suggest that the *P. aleyreanum* oil has selectivity to melanoma cells and thus this activity is probably related to a synergic effect, since b-elemene seems to be cytotoxic to all cancer cell types. **Financial Agencies:** CNPQ, CAPES, UFPA, FAPESPA. **References:** 1. Lago, J.H.G., Chen, A., Young, M.C.M., Guimarães, E.F., Oliveira, A., Kato, M.J., 2009. Prenylated benzoic acid derivatives from Piper aduncum L. and P. hostmannianum C. DC. (Piperaceae). Phytochemistry 24, 96-98. 2. Li, X., Wang, G., Zhao, J., Ding, H., Cunningham, C., Chen, F., Flynn, D.C., Reed, E., Li, Q.Q., 2005. Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. Cellular and Molecular Life Science 62, 894-904. 3. Li, Q.Q., Wang, G.D., Huang, F.R., Banda, M., Reed, E., 2010. Antineoplastic effect of betaelemene on prostate cancer cells and other types of solid tumour cells. J. of Pharmacy and Pharmacology. 62, 1018-1027.

10.008 Comparison of antitumor activity of *Blechnum occidentale* L. (Blechnaceae) between native and cultivated plants. Nonato FR[1], Ruiz ALTG[1], Silva EB[2], Oliveira LM[2], Veiga LF[3], Melo PS[3], Alencar SM[3], Carvalho JE[1] [1]CPQBA-Unicamp – Farmacologia e Toxicologia, [2]UEFS – Ciências Biológicas, [3]ESALQ-USP – Agroindústria, Alimentos e Nutrição

Introduction: Studies have shown that Brazilian ferns are responsible for a number of biological activities. Several species are shown to be endowed with antibiotic, antiulcer, antioxidant, analgesic, antinociceptive, anti-inflammatory, sedative and even anticonvulsant activities. *Blechnum* is a genus of between 150-220 species of ferns with a cosmopolitan distribution in the Blechnaceae family. Most are herbaceous plants, but a few species are tree ferns with stems up to 3 m tall. Some species of *Blechnum* are employed in folk medicine worldwide, so they becomeinterestingforpharmacologicalstudies. In order to evaluate the antitumor activity of *Blechnumoccidentale*, methanolic extract and its fractions from native and cultivated plants were tested. **Methods**: The native plants were collected in an urban remaining of Atlantic Florest in Salvador, Bahia, Brazil. Plant samples were cultivated in Experimental Unity Horto Florestal of Universidade Estadual de Feira de Santana, Bahia, Brazil. The underground portion of the plants (roots and stems) was crushed and dried and then were subjected to extraction with methanol followed by liquid-liquid extraction to obtain four fractions: hexane, chloroform, ethyl acetate and methanolic fractions. The study of antiproliferative activity *in vitro* was conducted in seven human tumor cell lines: U251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (drug-resistant ovarian), NCI-H460 (lung, no small cells), HT-29 (colon), PC-3 (prostate) and 786-0 (kidney). These results were expressed as GI_{50} (concentration that inhibits 50% of cell growth) and in order to compare all samples an arithmetic mean of GI_{50} was used. **Results and discussion:** The tests showed some difference of antitumor activity of the methanolic extract and its fractions between cultivated and native plants, based on GI_{50} mean value. The methanolic extract from the cultivated plant presented slightly more antiproliferative activity than native plant (mean GI_{50}>137.85 µg/mL for native plant and mean GI_{50} 128.63 µg/mL for cultivated plant. Subsequent tests exhibited more significant difference in activity: hexane fraction (mean GI_{50}>205.32 µg/mL for native plant and mean GI_{50} 32.26 µg/mL for cultivated plant); chloroform fraction (mean GI_{50}>5.47 µg/mL for native plant and mean GI_{50} 163.10 µg/mL for cultivated plant); ethyl acetate fraction (mean GI_{50}>132.26 µg/mL for native plant and mean GI_{50} 103.14 µg/mL for cultivated plant); and methanolic fraction (mean GI_{50}>85.53 µg/mL for native plant and mean GI_{50} 111.71 µg/mL for cultivated plant). **Conclusion**: These results suggested different chemical composition of the extracts and fractions, based on the production of distinct secondary metabolites in both different types of environment. Chemical analysis is being conducted at the present to elucidate the composition of the samples tested. **Financial support**: CNPq, CPQBA/UNICAMP

10.009 RNA interference with nNOS reinforces the IFN-γ injury in glioma cell lines. Resende FFB[1], Silva SS[1], Caldeira FMC[1], Pardo L[2], Stühmer W[2], Del Bel EA[3], Titze-de-Almeida R[1] [1]FAV-UnB – Tecnologias para Terapia Gênica, [2]Max-Planck-Institute – Molecular Biology of Neuronal Signals, [3]FORP-USP – Neurofisiologia e Biologia

Introduction: Glioma cells overexpress the neuronal nitric oxide synthase enzyme (nNOS). The NO synthase inhibitor Ng-nitro-L-arginina methyl ester (L-NAME) reduces the cancer cell proliferation in animal models of glioma. Those findings suggest a role for nNOS in tumor growth and metastasis. On the other hand, the cytokine interferon gamma (IFN-γ) also presents antitumoral activity on brain tumors. The present study was aimed to examine whether the injury caused by IFN-γ in glioma cells would be affected by an RNAi on nNOS enzyme. **Methods:** We first examined if an nNOS-targeted siRNA named siRNAnNOShum_4400 would cause nNOS knocking-down in three distinct glioma cell lines – U251MG, U87MG and U138MG. For that, glioma cells in culture were transfected with siRNAnNOShum_4400 or a negative control (37,5 nM) mixed with LIPOFECTAMINE 2000™. Changes in nNOS mRNA content were examined at three time-points: 4h, 24h, and 48h. We determined the mRNA content by RT-qPCR using SYBR Green™ dye assay chemistry (Applied Biosystem™). The fold decrease in mRNA content was expressed by the $2^{-\Delta\Delta CT}$ method. To explore the role of nNOS during IFN-γinjury, we silenced nNOS by using a shRNA expressing vector with pSilencerTM 3.1-H1 platform (pnNOShum4400). A negative control vector was included. Then we measured the effects on the viability of glioma cell U251MG by the MTT assay; absorbance was measured at 595 nM. Briefly, a 96-well plate was seeded in triplicate with 104 cells/well in 200µL of culture medium. The cells were injured with IFN-γ (25 ng/mL) and silenced from nNOS by shRNA expression vectors (0.2 µg). Effects on cell viability were measured at two different time-points, 24h or 48h. **Results and Discussions:** Changes in nNOS mRNA content in glioma cells transfected with siRNAnNOShum_4400 varied according to the cell lineage and time point tested. The U251MG cell line showed reduction in nNOS mRNA content at both time points 4h and 24h (0.88 fold and 0.61 fold, respectively). The lineage U87MG showed an nNOS fold decrease of 0.48 and 0.56 at the same time points. U138MG cells also showed nNOS knockdown, but only at 24h post transfection (0.62 fold). At 48 hours, we found an unexpected nNOS mRNA increased in all three cell lines, ranged to 1.37 fold (U87MG) up to 1.52 fold (U138MG). Regarding to cell viability, the nNOS shRNA expression vector pnNOShum4400 reinforced the injury caused by IFN-γ. Glioma cells transfected with 0.2 µg of the vector immediately after IFN-γ injury were 61% less viable at 24h ($P < 0.05$). The decrease in cell viability was aligned with the decrease in nNOS content by RNAi at the same time point. No positive effect between vector and IFN-γ was found at 48h, the time point with increased levels of nNOS mRNA. **Conclusions:** The data presented here suggest a role for nNOS in glioma cell defenses against the cytokine IFN-γ, an agent useful for brain tumor treatment. A further study might investigate whether the vector effects would also occur in animal models of cancer disease.

10.010 Toll-like receptor 4 (TLR4) regulates signaling proliferation on human melanoma cells. Souza MJ, Ribeiro-Pereira C, Barja-Fidalgo C UERJ – Biologia Celular

Introduction and objective: Toll-like receptors (TLRs) are important pattern recognition receptors which have roles in innate and adaptive immune responses. Moreover, there are increasing evidences that tumor cells express these receptors. Studies have reported that human melanoma cells express TLRs, including TLR4, although their functional activity has not been completely understood in those cells. In this study, we investigated the role of TLR4 on the proliferation and migration of two human melanoma cells lineage - MV3 (high metastatic melanoma) and SKMEL-28 (less metastatic than MV3 cell line) - and human melanocytes cells (NGM), as well as, the signaling pathways involved in these processes. **Material and Methods:** Human melanoma cells (MV3 and SKMEL-28) and melanocytes cells (NGM) were incubated in absence or presence of lipopolysaccharide (LPS) for 1 hour. Then, cellular proliferation was assessed by MTT assay. To evaluate cellular migration, the wound healing assay was performed. Whole cell extracts were obtained for immunoblotting assay. **Results and discussion:** TLR4 activation by LPS enhances the proliferation of the highly metastatic melanoma cells, MV3. However, the treatment with LPS was not able to modify neither SKMEL-28, nor melanocytes NGM cells proliferation. Increasing in MV3 cells proliferation induced by TLR4 activation on MV3 cells was followed by an augment in AKT phosphorylation and a decrease in ERK and p38 phosphorylation. In contrast, the treatment with LPS, for 1 hour, did not modify migration, a key process for tumor progression, of any melanoma cell (MV3 and SKMEL-28) or melanocytes. Taken together, our data suggest that, in contrast with normal or low metastatic cells, MV3 melanoma cells, which display a high metastatic profile, express TLR4, which can modulate signaling pathways involved in the proliferative process. The data and ongoing studies expect to contribute for a better understanding of cancer biology as well as in the search for new therapeutic targets. (Supported by: FAPERJ, Capes,CNPq).

10.011 Silencing EAG1 enhances temozolomide effects on glioblastoma cells in culture. Sales TT[1], Resende FFB[1], Rocha WS[1], Del Bel EA[2], Pardo L[3], Stühmer W[3], Titze-de-Almeida R[1] [1]FAV-UnB – Terapia Gênica, [2]FORP-USP – Neurofisiologia e Biologia Molecular, [3]Max-Planck-Institute – Molecular Biology of Neuronal Signals

Introduction: Growing evidence involves the ether a go-go 1 (EAG1) potassium channel in tumor growth and metastasis. This study focused on glioblastoma, one of the most lethal and treatment-resistant brain tumor. The standard therapy for patients with glioblastoma includes chemotherapy with temozolomide (TMZ) and the radiotherapy. However, the antineoplastic therapy can´t prolong the mean survival time of patients with glioma beyond 12 - 15 months. In the present study, we have evaluated whether Eag1 gene silencing would improve the effects of TMZ on glioma cells in culture. **Methods:** Glioblastoma cells U87MG were grown in a 96-well plate, in triplicate, with 10^4 cells/well in 200µL of culture medium (DMEM, Gibco™ with 10% FBS). Then the cells were transfected with a shRNA expressing vector named pKv10.1-3 (0.3µg), mixed with lipofectamine (Lipofectamine 2000 Transfection Reagent; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The vector pKv10.1-3 was built by using the pSilencerTM 3.1-H1 platform (Applied Biosystems, Carlsbad, CA, USA). The shRNAs target a previously described Eag1 mRNA sequence 5'-GTCCACTTGGTCCATGTCCAG-3' found in exon 8, nt 1793–1813. After transfection, cells were incubated for 24 h, at 37˚C with 95% humidity and an atmosphere with 5% CO_2. Then they received TMZ at 250 µM or 500 µM. Cell viability was quantitatively evaluated at the time point 72 h post transfection by colorimetric assay with MTT 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Invitrogen, Carlsbad, California, USA) at 595 nm. To test for intergroup differences, we applied one-way analysis of variance (ANOVA) followed by Tukey's post test. Differences were considered statistically significant at $p<0.05$. **Results:** We first found a dose-dependent decrease in glioma cell viability following TMZ treatment. Cell viability reduced to values of 50% or 70% according to each TMZ concentration tested, 250 µM or 500 µM, respectively ($p<0.05$). Indeed, the shRNA expressing vector pKv10.1-3 also showed ability to reduce the cell viability up to 45% ($p<0.05$). The association between the vector and TMZ caused the highest effects on glioma cells. The drop in cell viability reached 75% or 85%, according to each TMZ concentration tested (250 or 500 µM) ($p<0.05$). **Discussion:** Previous studies show Eag1 plays a role in cancer biology. Our study corroborates those findings, as Eag1 knocking-down by vector pKv10.1-3 caused a significant decrease in glioma cell viability. Also, TMZ caused the highest effect in glioma cells that were silenced from Eag1. This result suggests this channel would be a relevant player preserving the cell viability during TMZ injury. A further study would test this protocol in animal models of cancer, in order to explore whether Eag1 could be a target for a gene therapy based on RNAi. **Financial support:** CAPES, CNPq, FAP-DF.

10.012 Effects of the calcium channels blocker verapamil on surgically damaged liver under the biological curative amniotic membrane (homogenous) in rats: **Immunohistochemistry study**. Vilela-Goulart MG, Gomes MF, Bastos-Ramos WP CEBAPE-Unesp-São José dos Campos

Introduction: Calcium channels related cascades are intimately involved in deleteterious hepatic cells effects. Immunohistochemistry analysis was used to evaluate the influence of calcium channel blocker verapamil associated with the biological curative homogenous amniotic membrane (hAM) on the repairing of surgically damaged livers. **Methods**: Ninety six male adult rats (*Wistar*), were divided in four groups: 1)-LH group: control-rats with liver damage; 2)-hAM group: rats with liver damage plus hAM; 3)-V group: rats with liver damage plus verapamil; 4)-VhAM group: rats with liver damage, under verapamil and hAM treatment. hAM was obtained from 24 pregnant females. Surgeries were performed under ketamin-xylazine anesthesia, and hAM dressed the injured area. At the 10th, 20th, 30th and 40th days after the surgery, the animals were sacrificed and excised liver tissue to immunohistochemistry analysis with monoclonal PCNA antibody. **Resultsand Discussion**: It was shown the hAM transformation in an amniohepatic tissue, which is an evidence of biocompatibility. In addition, the hAM association to verapamil leads to an enhancement on PCNA expression. The results evidence an important protective effect of verapamil, which could be attributed to lowered intracellular calcium induced by the calcium blocker, since it has been demonstrated that as increase of the cytosolic calcium concentration can induce a variety of phenomena that lead to liver cell lesion and death. The protective response of amniotic membrane, not alone, but in the presence of verapamil could be attributed to the membrane property of enhancing wound healing in various tissues and liver. In conclusion, the amniotic membrane showed mitogenic activity. The verapamil stimulated liver regeneration and presented hepatoprotective property. The increase of PCNA expression is probably related to the increase of DNA strands synthesis during replication. Financial support by FAPESP (08656-3). Approved by Ethics Committee for Animal Use 0505/2008

10.013 Evaluation cytotoxic of compound thiophenic 6CN-10 in pancreatic carcinoma. Aquino ACQ[1], Carvalho MS[1], Aguiar ACV[1], Rocha HAO[2], Moura RO[3], Mendonça Junior FJB[3]
[1]UFRN – Biofísica e Farmacologia, [2]UFRN – Bioquímica, [3]UEPB – Ciências Biológicas

Introduction: Thiophene is a group of heterocyclic compounds that has been extensively studied worldwide, with several pharmacological activities such as analgesic, and antipyretic activities, adrenergic and more recently has been reported as an antitumor agent and anticancer. This work, we investigated the cytotoxic effect of the derivative of thiophene **6CN-10** with property antitumoral using cancer cell line of pancreatic carcinoma (**PANC**). **Methods:** The compound thiophenic **6CN-10** was synthesized and tested on the human cancer cell line **PANC** (pancreatic carcinoma). For comparative purposes, it used an anticancer drug used in clinic, doxorubicin (**DOX**), as positive control. The citotoxicity was evaluated by the MTT test. The compound previously dissolved in DMSO was diluted in DMEM to obtain the final concentrations (5, 10, 25 and 50 μM) and added in 96-well micro plates (100μL/well), with a cellular density of 5x10³ cells/well. The cells were incubated in the presence of compound 6CN-10 by 24, 48 and 72h at 37°C and 5% CO2, in triplicate. After each period, the supernatant was aspirated, was added 100μL of MTT (5 mg/ml in DMEM) and the microplates were replaced in an incubator for 4h. Moreover, the supernatant was aspirated and the formazan product was solubilized by the addiction of 100μL of ethanol. The plates were agitated during 15 minutes and the absorbance was read on spectrophotometer at a wavelength of 570nm. Statistical analysis were performed according to their means and standard errors and deviations were made from non-linear regression using Graph Pad Prism (version 6). **Results and Discussions:** It was observed that the compound **6CN-10** presented a cytotoxic effect in the line **PANC**, with a significant difference among the control group, the test compound **6CN-10** and the reference standard drug **DOX**, with the following percentages of cell proliferation, respectively: 100% control group, 85,8% **DOX** and for the compound **6CN-10** 45,9 %. As it shown, the compound **6CN-10** at lowest concentration of 5 μM was more effective at inhibiting cell proliferation when compared to **DOX** at the same concentration. Further investigation regarding possible molecular targets for the cytotoxic effect of the compound is needed. **Conclusion:** As it shown, the compound **6CN-10** was more effective in cytotoxic activity when it compared to doxorubicin (**DOX**) at lowest concentration of 5 μM. Further investigations regarding possible molecules targets for the cytotoxic effect of the compound are needed to emphasize the hypothesis that the molecular is candidate for the anticancer drug. **References:** Mohareb, R. M., Steroids, 77, 1551, 2012. Shchekotikhin, A. E. Bioorganic & Medicinal Chemistry, 17, 1861, 2009. R. Romagnoli, European Journal of Medicinal Chemistry, 45, 5781, 2010. **Financial Agencies and acknowledgments:** Laboratório de Farmacologia/UFRN Laboratório de Biotecnologia de Polímeros Naturais (BIOPOL)/UFRN Laboratório de Síntese e Vetorização de Moléculas/UEPB. CNPq

10.014 Production of melatonin by Glioma cell lines. Kinker GS[1], Marie SK[2], Oba-Shinjo SM[2], Muxel SM[1], Carvalho-Sousa CE[1], Fernandes PA[1], Markus RP[1] [1]IB-USP – Fisiologia, [2]FM-USP – Neurologia

Introduction: Gliomas, the most frequent primary brain tumors, are classified according to tumor differentiation, mitotic activity and grade of invasiveness. Challenges in the treatment of high-grade gliomas include the tumor heterogeneity, the inability of the drugs to reach all the neoplastic cells and the complexity of the tumor microenvironment. *In vitro* studies have shown that melatonin significantly reduces the migration and invasion of glioma cells and inhibits the expression of matrix metalloproteinase, suggesting a potential therapeutic application (Wang *et al.*, *J Pineal Res,* 53, 180, 2012). The aim of this work was to correlate the capacity of gliomas to synthesize/metabolize melatonin with the grade of tumor invasiveness. **Methods:** We analyzed the expression of genes that codify melatonin synthesis enzymes [arylalkylamine N-acetyltransferases (*AANAT*) and N-acetylserotonin O-methyltransferase (*ASMT*)] in human glioma samples. The relative expression of *AANAT* and *ASMT* was determined from a whole genome oligonucleotide microarray data set containing 55k transcripts. We then evaluated the activity of ASMT (which converts N-acetylserotonin, NAS, in melatonin) in glioma cell lines (U87MG, high invasiveness; T98G, medium invasiveness; HOG, low invasiveness). Cultures were incubated with increasing concentrations of NAS (10 nM – 0.1 mM) for 6 hours and the content of melatonin in the medium was determined (data are shown as mean ± SEM and correspond to the percentage of control mean). We also analyzed the expression of *CYP1B1*, which codifies for the enzyme that metabolizes melatonin in the brain, using real-time PCR. **Results:** No expression of *AANAT* was detected among the tumor samples; *ASMT* was detected in oligodendrogliomas and grade I-II astrocytomas, but not in grade IV glioblastomas. This result indicates that highly invasive tumors are not able to convert NAS in melatonin. After incubation with 0.01, 0.03 and 0.1 mM of NAS, the concentration of melatonin in the medium of HOG cells (15410 ± 887,6 N=3; 56842 ± 2274 N=3; 187714 ± 13810 N=3, respectively) was significantly higher compared to T98G cells (10566 ± 1007 N=6; 22390 ± 277.5 N=3; 50163 ± 553.1 N=3, respectively) and U87MG cells (9554 ± 788,1 N=6; 19617 ± 383.3 N=3; 44042 ± 993.5 N=3, respectively). The concentration of melatonin in the medium of T98G cells was significantly higher than U87MG, after incubation with 0.03 and 0.1 mM of NAS. The expression of *CYP1B1* by U87MG cells was approximately 30-fold higher than T98G cells and 2-fold higher than HOG cells. **Discussion:** Our data strongly suggest that non-invasive tumors are able to transform NAS in melatonin *in situ*, while higher invasive tumors probably metabolize melatonin in a faster manner. Financial support: FAPESP (2012/23915-1, 2010/52687-1), CNPq.